METHYL TERTIARY-BUTYL ETHER

CAS # 1634-04-4

ORAL RISK ASSESSMENT DOCUMENT

NSF International
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AUTHORS, PEER REVIEWERS, AND ACKNOWLEDGEMENTS

Author:
NSF Toxicology Services
1.800.NSF.MARK
NSF International
789 Dixboro Road
Ann Arbor, MI 48105

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Internal NSF Peer Reviewers:
Clif McLellan, M.S.
Gwen Ball, Ph.D.
Carolyn Gillilland, M.S.

EXECUTIVE SUMMARY

METHYL *t*-BUTYL ETHER – Oral Risk Assessment CAS # 1634-04-4			
PARAMETER	**LEVEL**	**UNITS**	**DERIVED**
$BMDL_{10}$ (10% Benchmark Dose Level)	32	mg/kg-day	From a chronic gavage study in rats
10^{-5} Cancer Risk Level	0.003	mg/kg-day	From a chronic gavage study in rats
Oral RfD (oral reference dose)	Not determined	mg/kg-day	Not applicable
TAC (total allowable concentration)	0.1	mg/L	For an adult drinking 2L water/day
SPAC (single product allowable concentration)	0.01	mg/L	For an adult drinking 2L water/day
STEL (short term exposure level)	Not Determined	mg/L	Not applicable

KEY STUDY	Belpoggi, F., M. Soffritti, and C. Maltoni. 1995. Methyl-tertiary-butyl ether (MTBE) - a gasoline additive - causes testicular and lympho-haematopoietic cancers in rats. Toxicol. Ind. Health. 11(2):119-149.
CRITICAL EFFECT(S)	Leydig (testicular interstitial) cell tumors in male rats and hemolymphoreticular leukemias/lymphomas (combined) in female rats
UNCERTAINTY FACTORS	There were no uncertainty factors applied, since a cancer risk assessment assuming a linear mode of action was performed.
TOXICITY SUMMARY	Increased liver weights, aspartate aminotransferase, blood urea nitrogen, cholesterol, and centrilobular hypertrophy were observed in rats administered methyl t-butyl ether via gavage for 28 days or more. Short-term and subchronic gavage exposures were associated with increased mean absolute and relative kidney weights in male rats and hyaline droplet formation in the renal proximal tubules. The increases in liver weight, liver-related clinical measurements, and centrilobular hypertrophy were likely due to an adaptive mechanism by the liver to metabolize methyl t-butyl ether, based on CYP450 induction data and the lack of reported non-neoplastic effects after chronic gavage exposures. However, the chronic non-neoplastic data were not available for review. Chronic gavage exposure was associated with an increase in Leydig cell tumors in male rats and leukemias/lymphomas (combined) in female rats. No standardized two-generation reproduction or developmental studies via the oral route were identified. Attempts to characterize the mode of action for the Leydig cell tumors in non-standardized reproduction studies revealed that single gavage doses at approximately 500 mg/kg-day resulted in reduced circulating testosterone immediately following dosing. At gavage doses of 1,200 mg/kg-day for 14 days, decreased circulating testosterone and luteinizing hormone, increased estradiol, and decreased testicular microsomal aromatase activity were observed in male rats. *In vivo* metabolism data indicate that oral exposure to methyl t-butyl ether for up to 28 days induces various CYP450 isozymes. Methyl t-butyl ether is oxidatively demethylated to t-butanol. In rodents, the biotransformation of t-butanol has been shown to yield 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid. No evidence of hepatic peroxisome proliferation was observed. All investigations on nephrotoxicity were consistent with α-2μ-globulin nephropathy, which was not considered relevant to humans. The weight of genotoxicity evidence suggests that methyl t-butyl ether has some genotoxic potential and there were insufficient data to support a non-genotoxic mode of action. Thus, a 10^{-5} cancer risk level for methyl t-butyl ether was extrapolated from the chronic gavage $BMDL_{10}$, which was essentially the same, whether based on the Leydig cell tumors in male rats (32 mg/kg-day) or on lymphatic tumors in female rats (36 mg/kg-day).
CONCLUSIONS	There are no chronic data in humans, but there is "*suggestive evidence of carcinogenic potential*" after gavage exposure to methyl t-butyl ether in rats. The drinking water action levels developed in this risk assessment are protective of public health, since they were calculated based on the tumor incidences observed in a chronic gavage study. Although the study was flawed, it was considered adequate for the purposes of risk assessment and more appropriate than using a chronic inhalation study with inhalation to oral route extrapolation to estimate a lifetime cancer risk from oral exposure to methyl t-butyl ether.

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **methyl t-butyl ether** in drinking water as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2007) or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2007). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993; U.S. EPA, 2002), which assumes that the threshold for these endpoints will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996; U.S. EPA, 2002) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED_{10} or $BMDL_{10}$ from benchmark dose programs) can be used (U.S. EPA, 2007a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 2005a).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70\ \text{kg}] - [\text{total contribution of other sources (mg/day)}]}{2\ \text{L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70\ \text{kg}}{2\ \text{L/day}} \times 0.2\ (\text{RSC})$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and

distribution system. In the absence of source data, a default multiple source factor of 10 is used. The multiple source factor accounts for the possibility that more than one product in the water and/or its distribution system could contribute the contaminant in question.

Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED_{10} of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to a level at or below the TAC under static conditions, or to a level at or below the SPAC under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a, U.S. EPA, 1999; U.S. EPA, 2003b). If there is sufficient evidence to use a non-linear model, the LED_{10} or $BMDL_{10}$, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED_{10} or $BMDL_{10}$ to zero is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10^{-6} to 10^{-4} is considered by the U.S. EPA to be safe and protective of public health (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2005) and 61 (2007), the TAC is set at the 10^{-5} risk level, and the SPAC is set at the 10^{-6} risk level. Use of a higher risk level is not ruled out, but would generally require documentation of a benefit to counteract the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2005) and 61 (2007). Application of these values to other exposure scenarios should be done with care and with a full understanding of the values derivation and the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation and require the use of additional uncertainty factors to protect public health.

The general guidelines for this risk assessment include those from the National Research Council (NRC, 1983) and from the Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a; 1997b). Other guidelines used in the development of this assessment may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), draft final Guidelines For Carcinogen Risk Assessment (U.S. EPA, 2003b), Guidelines for Carcinogen Risk Assessment (2005b), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998), A Review of the Reference Dose and Reference Concentration Process (U.S. EPA, 2002), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing Guidelines (U.S. EPA, 2007b).

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)
- Current Contents (as requested)

The literature search for this chemical was conducted on September 29, 2003 and updated on January 23, 2008. This document includes all relevant information retrieved as a result of those searches.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

Methyl t-butyl ether is an aliphatic dialkyl ether with synonyms of 2-methoxy-2-methylpropane; 2-methyl-2-methoxypropane; ether, tert-butyl methyl; MTBE; methyl 1,1-dimethylethyl ether; methyl tert-butyl ether; methyl tertiary-butyl ether; propane, 2-methoxy-2-methyl-; t-butyl methyl ether; tert-butyl methyl ether (ChemIDPlus, 2003). It has trade names of 3 D Concord, Driveron, HSDB 5487, and UN 2398 (IPCS, 1998). It has the following structure, and physical and chemical properties listed in Table 1:

Table 1. The physical and chemical properties of methyl t-butyl ether

Property	Data	Reference
Empirical Formula	$C_5H_{12}O$	OEHHA, 1999
CAS#	1634-04-4	OEHHA, 1999
Molecular Weight	88.15	OEHHA, 1999
Physical State and Color	colorless liquid at room temperature	IPCS, 1998
Melting Point	-109°C	OEHHA, 1999
Boiling Point	55.2°C	IPCS, 1998
Density	0.7404 at 20°C	IPCS, 1998
Vapor Pressure	33,500 Pa at 25°C	IPCS, 1998
Water Solubility	51 g/L at 25°C	OEHHA, 1999
Dissociation Constant (pK_a)	Not reported	
n-Octanol/Water Partition Coefficient (log K_{ow})	0.94-1.3[a] 1.43 (estimated)[b]	[a] IPCS, 1998 [b] http://esc.syrres.com
Henry's Law Constant (air/water partition)	5.87×10^{-4} atm-m^3/mole at 25°C	OEHHA, 1999

2.1 Organoleptic Properties

Methyl t-butyl ether has a terpene-like odor (IPCS, 1998). Individual variability in sensitivity to taste and odor make it difficult to identify odor and taste thresholds for methyl t-butyl ether in water (ECB, 2002). IPCS (1998) has reported that the taste threshold for methyl t-butyl ether in water is 134 ppb. OEHHA (1999) has cited various sources that report odor thresholds for methyl t-butyl ether in water of between 2.5 to 680 ppb. The U.S. EPA (1997) recommended a drinking water level of 20-40 ppb for methyl t-butyl ether, based on averting taste and odor. More recent data by Suffet et al. (2007) suggests that the odor threshold for methyl t-butyl ether in water is ≥ 15 ppb.

3.0 PRODUCTION AND USE

3.1 Production

Industrially, methyl t-butyl ether is derived from the catalytic reaction of methanol and isobutylene over an acidic ion-exchange resin catalyst such as sulfonated styrene cross-linked with divinyl benzene in the liquid phase at 38-93°C and 100-200 psi (IPCS, 1998). It can also be prepared from methanol, t-butanol, and diazomethane.

Methyl t-butyl ether is among the 50 highest production volume chemicals (IPCS, 1998). In 1999, total worldwide annual production of methyl t-butyl ether was about 21 million tons or 46.3 billion pounds (ECB, 2002). Methyl t-butyl ether is a high production volume chemical in the United States (U.S. EPA, 2007c) and European Union (2004).

3.2 Use

It is anticipated that the use of methyl t-butyl ether will continue to increase (IPCS, 1998). North America is the largest consumer of methyl t-butyl ether, accounting for about two-thirds of the

world's annual use (IPCS, 1998). In 1996, the US was the world's largest consumer of methyl t-butyl ether with a usage of 10.6 million tons (12.2 billion pounds) per year.

The major use of methyl t-butyl ether is as an oxygenated additive in gasoline, in which it is blended at 2 to 11.5% by volume (ECB, 2002). IPCS (1998) reports that methyl t-butyl ether has been added to gasoline in concentrations up to 17% by volume. Only a minor amount is used for other purposes, such as solvent instead of diethyl ether or diisopropyl ether in both the chemical and pharmaceutical industry and laboratories (ECB, 2002). Approximately 25% of gasoline in the USA is blended with methyl t-butyl ether (IPCS, 1998). Methyl t-butyl ether is almost exclusively used to provide both octane enhancement and an increase in the oxygen content of gasoline. No approved uses for methyl t-butyl ether as a direct or indirect food additive were identified under Title 21 of the U.S. Code of Federal Regulations (U.S. FDA, 2007).

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

Sorption/desorption, including purge and trap systems, and headspace procedures have been used to prepare water for analysis of methyl t-butyl ether (IPCS, 1998). The analytical methods for methyl t-butyl ether in water have been reviewed by IPCS (1998). These methods include the static headspace procedure using gas chromatography with photoionization detection (GC-PID) with a detection limit of 10.8 $\mu g/m^3$ and the purge and trap procedure using gas chromatography-mass spectrometry with detection limits ranging from 0.06 to 5 µg/L. NSF International uses U.S. EPA (1995) method 502.2 employing gas chromatography for volatile compounds to detect methyl t-butyl ether as an extractant from drinking water system components tested to NSF/ANSI Standard 61 (2007). The reporting limit is 0.5 µg/L.

4.2 Analysis in Biological Matrices

Methyl t-butyl ether is analyzed in biological matrices generally by gas chromatography, using a range of capillary columns and detector systems suited to the specific matrix (IPCS, 1998).

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

Methyl t-butyl ether does not occur naturally in the environment (IPCS, 1998). Groundwater may become contaminated with methyl t-butyl ether through leaking underground storage tanks or spillage from overfilling of the storage tanks (ECB, 2002). In the USA, methyl t-butyl ether has been detected in storm water, surface water, including streams, rivers, and reservoirs, groundwater, and drinking water (IPCS, 1998). Methyl t-butyl ether is infrequently detected in public drinking-water systems from groundwater. In all but three out of 51 systems in which it was reported, the concentration was ≤20 µg/L. There are inadequate data to characterize the concentration of methyl t-butyl ether in public drinking-water systems from surface water. Methyl t-butyl ether has been found at high levels (i.e. ≥1,000 µg/L) in a few private wells used for drinking water (IPCS, 1998). Methyl t-butyl ether has been detected as an extractant from

drinking water system components tested to NSF/ANSI 61 (2007) at normalized concentrations up to 0.2 mg/L.

Workers with potential exposure to methyl t-butyl ether include those involved in the production, distribution, and use of methyl t-butyl ether and methyl t-butyl ether-containing gasoline, including service station attendants and mechanics (IPCS, 1998). The sources of industrial occupational exposure to methyl t-butyl ether have been reviewed by ECB (2002) and include individuals involved in the production, formulation, transportation, or distribution of methyl t-butyl ether. These exposures include personnel employed at service stations, those involved in maintenance operations and automotive repairs, and individuals in the chemical or pharmaceutical industries in which methyl t-butyl ether is used as a solvent. Exposure of the public to methyl t-butyl ether can be principally by inhalation of fumes while refueling motor vehicles and drinking contaminated water (McGregor, 2006). Maximum internal doses resulting from such exposures are unlikely to exceed 0.05 mg/kg-day and will normally be very much lower.

5.2 Sources of Environmental Exposure

Methyl t-butyl ether may enter the environment during all phases of the petroleum fuel cycle (IPCS, 1998). Sources include auto emissions, evaporative losses from gasoline stations and vehicles, storage tank releases, pipeline leaks, other accidental spills, and refinery stack releases. Annual estimates of methyl t-butyl ether mass releases to the environment from all potential sources have not been reported in the scientific literature. However, releases from storage tanks, vehicular emissions, and evaporative losses from gasoline stations and vehicles are perceived to be important sources.

Concentrations of methyl t-butyl ether detected in storm water ranged from 0.2 to 8.7 µg/L with a median of less than 1.0 µg/L. For streams, rivers, and reservoirs, the range of detection was from 0.2 to 30 µg/L, and the range of medians for several studies was 0.24 to 7.75 µg/L. Methyl t-butyl ether has generally not been detected in deeper groundwater or in shallow groundwater in agricultural areas. When detected, the concentration is less than 2.0 µg/L. Methyl t-butyl ether is more frequently found in shallow groundwater (top 5-10 feet of these aquifers) in urban areas. In this setting, the concentrations range from less than 0.2 µg/L to 23 mg/L, with a median value below 0.2 µg/L (IPCS, 1998).

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

Numerous studies investigating the kinetics and metabolism of methyl t-butyl ether in humans and laboratory animals are available. These data have been reviewed by several regulatory organizations, including European Chemicals Bureau (ECB, 2002), Office of Environmental Health Hazard Assessment of the California EPA (OEHHA, 1999), the International Programme on Chemical Safety of the World Health Organization (IPCS, 1998), the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1992). Several review articles on these data are also available in the scientific literature.

Methyl t-butyl ether was absorbed into the blood of human volunteers who rapidly drank 2.8 mg methyl t-butyl ether in 250 mL Gatorade (Prah et al., 2004). Mean blood levels of methyl t-butyl ether peaked at 0.17 µmol/L between 15 and 30 minutes following administration and declined to at or below the detection limit (0.05 µmol/L) at the 24-hour sampling period. In human volunteers who rapidly drank 6.7 µl methyl t-butyl ether in "about 5 mg" of lemon-lime solution, peak blood levels of methyl t-butyl ether ranged from 5 to 15 ng/ml (0.06-0.17 µmol/l) (ECB, 2002).

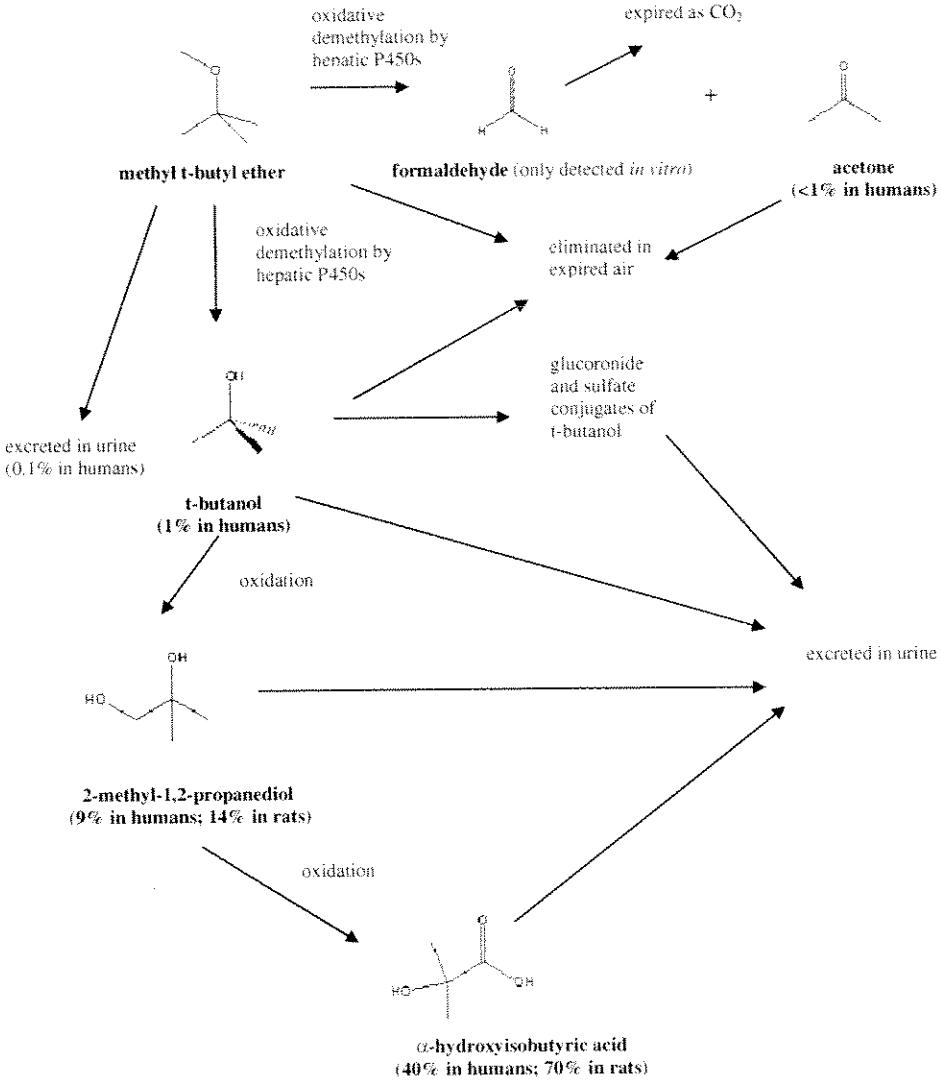
In rodents, methyl t-butyl ether is well absorbed and distributed following oral administration (IPCS, 1998). Rapid and complete absorption across the gastrointestinal tract was observed in rats administered methyl t-butyl ether via gavage at 40 mg/kg (ECB, 2002). At 400 mg/kg oral exposure in rats, the percentage of total absorbed dose eliminated in expired air increased with a corresponding decrease in the percentage eliminated in urine, indicating a saturation of metabolism (IPCS, 1998).

In vivo studies on the metabolism of methyl t-butyl ether in humans and rats indicate qualitatively similar overall metabolism (ECB, 2002). Methyl t-butyl ether is oxidatively demethylated by microsomal enzymes to t-butanol and formaldehyde, but the latter has only been shown *in vitro*. In rodents, the biotransformation of t-butanol has been shown to yield 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid (Figure 1).

The cytochrome P450-mediated biotransformation of methyl t-butyl ether has been explored in several *in vitro* studies with liver microsomes from humans, rats, and mice (ECB, 2002). Metabolism of methyl t-butyl ether by rat liver microsomes produced equivalent amounts of formaldehyde and t-butanol, and data strongly suggest that when expressed, CYP2B1 is the major enzyme involved in methyl t-butyl ether demethylation and that CYP2E1 may have a minor role.

Since these kinetic and metabolism data for methyl t-butyl ether in humans and laboratory animals have been reviewed previously, the current review focuses on only the new oral data since these reviews. Recent data confirm that methyl t-butyl ether is rapidly absorbed following oral administration. Approximately 30% of administered dose in humans was cleared by exhalation as unchanged methyl t-butyl ether and as t-butanol within 10-20 min. Less than 0.1% of the administered dose was recovered in expired air as acetone. Approximately 50% of the administered dose in humans was eliminated in the urine as unchanged methyl t-butyl ether (~0.1%), t-butanol (~1%), 2-methyl-1,2-propanediol (~9%), and 2-hydroxyisobutyrate (~40%).

Figure 1. Proposed metabolic scheme of methyl t-butyl ether



6.1 Absorption

Previous data in humans or laboratory animals demonstrate that methyl t-butyl ether is rapidly absorbed following oral administration. Recent data by Prah et al. (2004), Amberg et al. (2001), and Dekant et al. (2001) confirm this observation. Methyl t-butyl ether was rapidly absorbed

from the gastrointestinal tract and a significant part of the administered dose was transferred into blood of human volunteers ingesting methyl t-butyl ether in water or Gatorade. No other recent data regarding the absorption of methyl t-butyl ether following oral exposure in humans or laboratory animals were identified.

6.2 Distribution

Recent data regarding the distribution of methyl t-butyl ether after oral exposure were limited to the measurement of methyl t-butyl ether and one of its metabolites, t-butanol, in blood after oral ingestion in human volunteers.

Fourteen healthy male volunteers ingested 2.8 mg methyl t-butyl ether (unspecified purity) in 250 mL Gatorade (Prah et al., 2004). Gatorade was used as the vehicle to mask the unpleasant taste of methyl t-butyl ether. Blood samples were collected after 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, and 1,440 minutes. Mean levels of methyl t-butyl ether and t-butanol in the blood were determined using gas chromatography/mass spectrometry. The plasma half-life of methyl t-butyl ether was determined. The area under the plasma concentration versus time curve was estimated for methyl t-butyl ether alone and for methyl t-butyl ether plus t-butanol.

Mean blood levels of methyl t-butyl ether peaked at 0.17 µmol/L between 15 and 30 minutes following administration and declined to at or below the detection limit (0.05 µmol/L) at the 24-hour sampling period. Blood levels of t-butanol peaked at 0.23 µmol/L at the 45-minute sampling period and did not return to pre-exposure levels by the 24-hour sampling period. Elimination of methyl t-butyl ether from the blood was best characterized by a three-compartment model. The mean half-life for methyl t-butyl ether elimination from the blood in the first, second, and third phases was 14.9, 102.0, and 417.3 minutes, respectively. The mean area under the plasma concentration versus time curve was estimated to be 1,682 µmol/hr/L for methyl t-butyl ether alone, 20,025 µmol/hr/L for t-butanol, and 10,854 µmol/hr/L for methyl t-butyl ether and t-butanol combined. The mean area under the curve ratio of t-butanol to methyl t-butyl ether was 13.1 in the blood. Since this study also included the dermal and inhalation routes of exposure, the study authors suggested that these pharmacokinetic estimates were useful in constructing a physiologically-based pharmacokinetic model for methyl t-butyl ether in humans across different routes of administration.

Three human volunteers per sex and dose ingested 0, 5, or 15 mg ^{13}C-methyl t-butyl ether in 100 mL water (Amberg et al., 2001; Dekant et al., 2001). Blood samples were collected at 60-minute intervals for the first four hours and at 120-minute intervals thereafter until 12 hours. A final blood sample was collected 24 hours after administration.

At 5 mg, the maximum concentration in the blood averaged 0.10 µM, and these concentrations were obtained with the first blood samples, which were taken after one hour. Elimination of methyl t-butyl ether from the blood occurred in three phases, and the mean half-life of each phase was 0.8, 1.8, and 8.1 hours. Mean blood concentrations of t-butanol were 1.82 uM. The mean terminal half-life of t-butanol clearance from the blood was 8.1 hours. Levels of methyl t-butyl ether and t-butanol in blood declined to at or near the limit of detection at the 12- and 24-hour sampling times, respectively.

At 15 mg, the maximum concentration in the blood, which was reached after one hour, averaged 0.69 µM. Elimination of methyl t-butyl ether from the blood occurred in three phases, and the mean half-life of each phase was 0.7, 1.2, and 3.7 hours. Mean blood concentrations of t-butanol were 0.45 uM. The mean terminal half-life of t-butanol clearance from the blood was 8.5 hours.

6.3 Metabolism

6.3.1 Humans

The metabolism of methyl t-butyl ether was studied in three human volunteers per sex and dose after ingestion of 0, 5, or 15 mg ^{13}C-methyl t-butyl ether in 100 mL water (Amberg et al., 2001; Dekant et al., 2001). Mass spectrometry was used to identify urinary metabolites in urine samples collected at 6-hour intervals for 96 hours. At 5 and 15 mg, 46% and 49%, respectively, of the administered dose was eliminated in the urine as unchanged methyl t-butyl ether, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate. At 5 mg, unchanged methyl t-butyl ether, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate comprised 0.01, 1, 9, and 36% of the administered dose, respectively. At 15 mg, unchanged methyl t-butyl ether, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate comprised 0.1, 1, 8, and 40% of the administered dose, respectively. Hepatic first-pass metabolism was not observed. The authors concluded that the metabolic pathway for methyl t-butyl ether after oral exposure was identical to concurrently conducted inhalation exposure studies.

The metabolism of methyl t-butyl ether was studied in a panel of 12 human liver microsomes isolated from nine male and two female donors (Le Gal et al., 2001). The human liver microsomes metabolized methyl t-butyl ether into t-butanol and formaldehyde. The mean Michaelis-Menten constant (Km), which describes the catalytic power of an enzyme or rate of a reaction catalyzed by an enzyme, was determined. The mean apparent Km(1) was determined to be 0.25 mM, which was considered low by the study authors, and the mean apparent Km(2) was 2.9 mM, which was considered high. The study authors concluded that kinetic data, along with the results from correlation studies and chemical inhibition studies, support the assertion that the major enzyme involved in methyl t-butyl ether metabolism is CYP2A6, with a minor contribution of CYP3A4 at low substrate concentration.

6.3.2 Laboratory Animals

Williams and Borghoff (2000) investigated the hypothesis that methyl t-butyl ether-induced decrease in serum testosterone levels in male rats may be due in part to the ability of methyl t-butyl ether to induce the metabolism of endogenous testosterone and, hence, enhance its clearance. Fifteen male Sprague-Dawley rats per dose were administered 0 or 1,500 mg/kg-day methyl t-butyl ether (> 99.9% purity in corn oil) via gavage for 15 days. In a second experiment, fifteen male Sprague-Dawley rats per dose were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day methyl t-butyl ether (> 99.9% purity in corn oil) via gavage for 28 days. At study termination, the rats were sacrificed, body and liver weights were determined, and hepatic microsomes were isolated for measurement of CYP450 activity. Testosterone hydroxylase activities of hepatic microsomes, which were used as markers for CYP450 enzyme activities, were also assessed. These enzymes included 2-α-, 2-β-, 6-β-, 7-α-, 16-α-, and 17-β-

hydroxytestosterone. The activities of p-nitrophenol and UDP-glucuronosyltransferase were also assessed to evaluate the mechanism of centrilobular hypertrophy observed in rodents after repeated methyl t-butyl ether exposures. The formation of formaldehyde, a metabolite of methyl t-butyl ether, was also measured.

After 15 days, total hepatic microsomal cytochrome CYP450 was increased 1.3-fold in rats treated with 1,500 mg/kg-day methyl t-butyl ether. CYP1A1/2, CYP2A1, CYP2E1, and CYP2B1/2 activities were increased 1.5-, 2.4-, 2.3-, and 6.5-fold, respectively, at 1,500 mg/kg-day after 15 days. 7-α-hydroxytestosterone was statistically increased by 2.4-fold compared to controls.

After 28 days, total hepatic microsomal cytochrome CYP450 was not statistically different compared to control. At 1,000 mg/kg-day after 28 days, a statistical increase in mean relative liver weight (10-14%, not further specified) and a 2.0-fold increase in CYP2B1/2 were observed compared to controls.

After 28 days at 1,500 mg/kg-day, a statistical increase in mean relative liver weight (10-14%, not further specified) was observed. CYP 2B1/2, CYP2E1, CYP3A1/2, and UDP-glucuronosyltransferase activities were statistically increased by 2.9-, 2.0-, 2.1-, and 1.7-fold respectively, compared to controls. 6-β-hydroxytestosterone was statistically increased by 2.1-fold compared to controls. UDP-glucuronosyltransferase was statistically increased compared to controls. Formaldehyde production was statistically increased compared to controls at 1,500 mg/kg-day after 28 days. Methyl t-butyl ether also induced its own metabolism 2.1-fold at 1,500 mg/kg-day after 28 days, and the authors noted that this effect was consistent with the induction of CYP2E1 and CYP2B1. It should be noted that mean body weight was reduced by 12% compared to controls at 1,500 mg/kg-day after 28 days.

The study authors concluded that methyl t-butyl ether induced mild increases in testosterone hydroxylase enzymes. Further, the increase in UDP-glucuronosyltransferase was consistent with the centrilobular hypertrophy observed in rodents after repeated methyl t-butyl ether exposures. The decrease in serum testosterone observed following methyl t-butyl ether administration may be the result of enhanced testosterone metabolism and subsequent clearance. However, the authors stated that the most pronounced effects were observed at the high dose of 1,500 mg/kg-day, at which clinical signs of toxicity and reduced body weight (12%) were also observed. The authors further noted that since the increases in testosterone hydroxylase enzyme activities were generally mild, the hypothalamus-pituitary hormonal feedback loop could be expected to compensate for mild reductions in circulating testosterone *in vivo*.

Eight female B6C3F$_1$ mice per dose were given methyl t-butyl ether (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Eighteen hours after the last dose, the mice were sacrificed and hepatocyte cytochromes were isolated. Methyl t-butyl ether induced a statistical increase (37%) in total hepatic cytochrome P450 content, a 9-fold increase in hepatic 7-pentoxy-resorufin-*O*-dealkylase activity (a CYP2B marker) and a 2-fold increase in hepatic 7-ethoxy-resorufin-*O*-deethylase activity compared to controls.

6.4 Elimination/Excretion

The elimination of methyl t-butyl ether and t-butanol in expired air was investigated in seven healthy male volunteers who ingested 2.8 mg methyl t-butyl ether (unspecified purity) in 250 mL Gatorade (Prah et al., 2004). Gatorade was used as the vehicle to mask the unpleasant taste of methyl t-butyl ether. Exhaled air samples were collected after 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, and 1,440 minutes. Mean levels of methyl t-butyl ether and t-butanol in exhaled air were determined using gas chromatography/mass spectrometry.

Elimination of methyl t-butyl ether from expired air was best characterized by a three-compartment model. The mean half-life for methyl t-butyl ether in expired air in the first, second, and third phases was 13.0, 63.1, and 254.0 minutes, respectively. The mean area under the curve ratio of t-butanol to methyl t-butyl ether was 0.175 in exhaled air. Since this study also included the dermal and inhalation routes of exposure, the study authors suggested that these pharmacokinetic estimates were useful in constructing a physiologically-based pharmacokinetic model for methyl t-butyl ether in humans across different routes of administration.

The urinary elimination of methyl t-butyl ether was examined in three healthy human volunteers per sex administered 5 and 15 mg ^{13}C-methyl t-butyl ether (> 98% purity) in spiked tap water samples (Amberg et al., 2001). The different doses were administered four weeks apart. Urine samples were collected for 96 hours after administration in six hour intervals, and blood samples were taken in 60-minute intervals up to four hours, then at 120-minute intervals up to 12 hours, and ultimately at 24 hours. Methyl t-butyl ether and t-butanol concentrations in blood were determined. Urine metabolites, including the parent compound, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate were quantified.

At 5 and 15 mg/kg, 46% and 49%, respectively, of the administered dose was eliminated in the urine as unchanged methyl t-butyl ether, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate. The authors concluded that the kinetics of excretion after oral exposure were identical to concurrently conducted inhalation exposure studies.

In the same experiment, the respiratory elimination of methyl t-butyl ether was examined in three healthy male volunteers administered 15 mg ^{13}C-methyl t-butyl ether (> 98% purity) in 100 mL tap water samples (Amberg et al., 2001). Approximately 30% of the methyl t-butyl ether dose was cleared by exhalation as unchanged methyl t-butyl ether and as t-butanol. Methyl t-butyl ether exhalation was rapid and maximum concentrations of 100 nM in exhaled air were achieved within 10-20 min. Less than 0.1% of the administered dose was recovered in expired air as ^{13}C-acetone. The study authors concluded that the results indicate that the biotransformation and excretion of methyl t-butyl ether after oral exposure is similar to inhalation exposure and suggested the absence of a significant first-pass metabolism of methyl t-butyl ether in the liver after oral administration.

6.5 Physiologically-based pharmacokinetic models

Although several physiologically-based pharmacokinetic models have been constructed to model the behavior of inhaled methyl t-butyl ether, models describing the behavior of methyl t-butyl

ether after oral exposure are limited and usually include multiple exposure routes. Kim et al. (2007) developed a multiple-route (oral, inhalation and dermal) nine-compartment model of methyl t-butyl ether and t-butanol in humans based on blood measurements of these compounds. Borghoff et al. (1996) developed a multiple-route (oral, inhalation and intravenous) seven-compartment model of methyl t-butyl ether and t-butanol in F344 rats.

7.0 EFFECTS ON HUMANS

7.1 Case Reports

No recent case reports regarding oral exposure to methyl t-butyl ether were identified.

7.2 Epidemiological Studies

Epidemiological studies of human populations exposed under occupational as well as non-occupational conditions, and experimental studies of human volunteers exposed under controlled conditions, have not been able to identify a basis for headache, eye and nose irritation, cough, nausea, dizziness, and disorientation reported by consumers in some areas as a result of fueling with gasoline (IPCS, 1998). Although results are mixed, IPCS (1998) suggested that community studies conducted in Alaska, New Jersey, Connecticut, and Wisconsin provided limited or no evidence of an association between methyl t-butyl ether exposure and the prevalence of health complaints.

In controlled experimental studies on adult volunteers exposed in inhalation chambers to methyl t-butyl ether at concentrations ranging from 5.0 mg/m^3 (1.4 ppm) to 270 mg/m^3 (75 ppm), there were no evident effects on either subjective reports of symptoms or objective indicators of irritation or other effects up to 180 mg/m^3 (50 ppm) for up to two hours (IPCS, 1998). Thus, it appears unlikely that methyl t-butyl ether alone induces adverse acute health effects in the general population after inhalation exposure. However, the potential effects of mixtures of gasoline and methyl t-butyl ether, and the manner in which most persons are exposed to methyl t-butyl ether in conjunction with the use of oxygenated fuels, have not been examined experimentally or through prospective epidemiological methods.

8.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

Numerous regulatory organizations, including European Chemicals Bureau (ECB, 2002), Office of Environmental Health Hazard Assessment of the California EPA (OEHHA, 1999), the International Programme on Chemical Safety of the World Health Organization (IPCS, 1998), the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1992) have critically reviewed the studies in laboratory animals for methyl t-butyl ether. This section includes only the oral studies for methyl t-butyl ether, due to their significance in the development of lifetime drinking water levels for methyl t-butyl ether, since studies by the inhalation and/or dermal routes have been critically reviewed elsewhere.

No evidence of hepatic peroxisome proliferation was observed in male rats administered methyl t-butyl ether via gavage at 800 mg/kg-day for 14 days, but increased mean relative liver weight and minimal-to-moderate centrilobular hypertrophy were observed in male rats administered methyl t-butyl ether via gavage at 1,000 mg/kg-day and above for 28 days. In male and female rats administered high doses of methyl t-butyl ether (1,250 mg/kg-day) via gavage for 28 days, statistically increased cholesterol levels of at least 20% compared to controls were observed.

Short-term and subchronic gavage exposures to methyl t-butyl ether were associated with increased mean absolute and relative kidney weights in male rats accompanied by hyaline droplet formation in the renal proximal tubules. In rats administered methyl t-butyl ether at 100 mg/kg-day and above via gavage for 13 weeks, statistically increased mean blood urea nitrogen levels of at least 15% compared to controls were observed. Chronic gavage exposure to methyl t-butyl ether was associated with an increase in Leydig cell tumors in male rats and leukemias/lymphomas (combined) in female rats.

8.1 Limited-Exposure Effects

Methyl t-butyl ether was found to be irritating to the eyes and skin of rabbits, but did not induce skin sensitization in guinea pigs.

8.1.1 Irritation and Sensitization Studies

Following the application of 0.5 mL of neat methyl t-butyl ether to the intact and abraded skin of six rabbits for 24 hours, a primary irritation index of 3.36 was reported, which was considered "moderately" irritating to skin (IPCS, 1998). Moderate erythema and edema were observed. Effects were slightly more pronounced on abraded skin. In mice, methyl t-butyl ether can induce slight to severe respiratory irritation following inhalation of 300 to 30,000 mg/m^3, respectively. A 1% induction and challenge concentration of methyl t-butyl ether did not induce skin sensitization in twenty guinea pigs (IPCS, 1998).

8.1.2 Ocular Exposure Studies

Methyl t-butyl ether was irritating to the eyes of rabbits and caused mild, but reversible, changes (IPCS, 1998).

8.2 Single-Exposure Studies

The oral (gavage) LD_{50} for methyl t-butyl ether is approximately 3,800 mg/kg in rats (IPCS, 1998) and 4,000 in mice (OEHHA, 1999). Signs of intoxication after a single oral lethal dose consisted of central nervous system depression, ataxia, labored respiration, and death.

8.3 Short-Term Exposure Studies

8.3.1 Three-Day Gavage Study In Female B6C3F$_1$ Mice

Eight female B6C3F$_1$ mice per dose were given methyl t-butyl ether (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Eighteen hours after the last dose, the mice were sacrificed and hepatocytes were isolated for measurement of hepatocyte proliferation *in vitro*, expressed as the amount of 5-bromo-2'-deoxyuridine incorporation into hepatocyte nuclei. The hepatic labeling index was calculated by dividing the number of labeled nuclei by the total number of nuclei and multiplying by 100. Body weight and absolute and relative liver weights were also measured. Body and liver weights were not affected by treatment, but methyl t-butyl ether induced a statistical increase in the hepatocyte labeling index of 6.5% compared to 2.5% in controls.

8.3.2 Fourteen-Day Gavage Study In Male And Female Sprague-Dawley Rats

Ten Sprague-Dawley rats per sex and dose were administered 0, 357, 714, 1,071, or 1,428 mg/kg-day methyl t-butyl ether (99.95% purity in corn oil) by gavage for 14 days (Robinson et al., 1990). The high dose was selected because it was 37% of the LD_{50}. Rats were housed separately by sex and food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Food and water consumption were measured throughout the study at unspecified intervals. Body weight was measured on Days 0, 4, 6, and 14. Hematology parameters and clinical chemistry were conducted on all rats at study termination. Hematology included hematocrit, hemoglobin, mean corpuscular volume, erythrocytes, leukocytes, differential leukocytes, and reticulocytes. Clinical chemistry included glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, cholesterol, calcium, and phosphorus. Brain, liver, spleen, lung, thymus, kidney, adrenal, heart, ovary, and testes weights were measured at study termination, and relative organ-to- body-weight ratios were calculated. Gross necropsies were conducted on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and included unspecified "major organs". If target histopathological organs were identified, these organs were also examined histologically in the remaining dose groups.

At 357 mg/kg-day, two males died, but the deaths were attributed to the gavage treatment. Diarrhea was observed in treated rats. Mean creatinine was statistically increased by 16% in males compared to controls. Mean absolute (15%) and relative (16%) lung weights were statistically lower in females compared to controls.

At 714 mg/kg-day, diarrhea and statistically reduced food intake (unspecified magnitude) were observed in males compared to controls. Mean hemoglobin (6%), hematocrit (4%), differential lymphocytes (6%), and creatinine (16%) were statistically increased in males compared to controls. Mean alanine aminotransferase (21%) and cholesterol (22%) were statistically increased and mean serum calcium (6%) was statistically decreased in females compared to controls. Mean absolute (11%) and relative (11%) lung weights were statistically lower in females compared to controls. Mean absolute (12%) and relative (9%) lung weights were statistically lower in males compared to controls.

At 1,071 mg/kg-day, diarrhea was observed in treated rats. Mean erythrocytes (6%), hemoglobin (6%), aspartate aminotransferase (43%), and lactate dehydrogenase (78%) were statistically increased, and mean differential monocytes (33%) were statistically decreased in males compared to controls. Mean cholesterol (34%) was statistically increased in females compared to controls. Mean absolute (14%) and relative (11%) lung weights were statistically lower in females compared to controls.

At 1,428 mg/kg-day, two males and two females died, but the deaths were attributed to gavage. Diarrhea and profound but transient (< two hours) anesthesia were observed after dosing in male and female rats. Statistically reduced food intake (unspecified magnitude) was observed in females compared to controls. Statistically reduced mean terminal body weight of 10% was observed in females compared to controls. Mean erythrocytes (7%), blood urea nitrogen (14%), aspartate aminotransferase (38%), cholesterol (37%), and lactate dehydrogenase (63%) were statistically increased, and mean differential monocytes (33%) were statistically decreased in males compared to controls. Mean glucose (15%) was statistically increased and mean blood urea nitrogen (27%) and creatinine (20%) were statistically decreased in females compared to controls. Mean absolute (22%) and relative (15%) lung weights were statistically lower in females compared to controls. Mean absolute spleen (18%) and mean absolute (20%) and relative thymus (27%) weights were statistically lower in females compared to controls. Mean relative kidney (8%) and brain (9%) weights were statistically higher in females compared to controls. The incidence of hyaline droplet nephropathy in the renal tubules was "moderately" increased in dosed male rats, but no further details were provided, with the exception that increased hyaline droplets within the cytoplasm of proximal tubular epithelial cells were noted in 7/8 (88%) high-dose males compared with 2/5 (40%) controls.

8.3.3 Fourteen-Day Gavage Studies In Male Sprague-Dawley Rats

In a 14-day gavage study, de Peyster at al. (2003) examined whether methyl t-butyl ether exposure could induce hepatic peroxisome proliferation, since other chemicals that cause Leydig cell tumors in rats were also shown to induce peroxisome proliferation. Six male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0 or 800 mg/kg-day via gavage for 14 days. Positive control rats were administered gemfibrozil via the diet. Hepatic peroxisomes were isolated from liver sections and processed for peroxisomal β-oxidation and examined with an electron microscope. Terminal blood samples were collected for measurement of cholesterol, triglyceride, alanine aminotransferase, and aspartate aminotransferease. Liver weights were measured, and relative liver-to-body-weight ratios were calculated. According to the study authors, there were no statistical differences between treated and vehicle control rats, but not all of the data were provided. It should be noted that although the methodology stated that methyl t-butyl ether doses of 800 mg/kg-day were administered, the results section indicated that methyl t-butyl ether doses were 1,000 mg/kg-day.

Ten male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0 or 1,200 mg/kg-day for 14 days (de Peyster et al., 2003). This dose was determined in previous experiments to lower circulating testosterone levels without affecting body weight. Liver, testes, accessory sex organs (unspecified), and brain weights were

measured. Total protein content and P450 content in hepatic microsomes was determined, and hepatic microsomal aromatase activity was measured.

In rats treated with 1,200 mg/kg-day methyl t-butyl ether, a statistical increase in mean relative liver weight of 15% was observed compared to controls. Although hepatic P450 content was comparable to controls, hepatic microsomal aromatase activity was decreased by 36% compared to controls.

8.3.4 Fifteen-Day Gavage Study In Male Sprague-Dawley Rats

Fifteen male Sprague-Dawley rats per dose were administered 0 or 1,500 mg/kg-day methyl t-butyl ether (> 99.9% purity in corn oil) via gavage for 15 days (Williams and Borghoff, 2000; Williams et al., 2000). At study termination, the rats were sacrificed, body, adrenal, kidney, epididymides, testes, prostate, pituitary gland, seminal vesicle, and liver weights were determined, and histopathological examination of the liver, kidneys, testes, and adrenals was conducted.

There were no treatment-related deaths. Deaths attributed to gavage, which were confirmed at necropsy, were primarily limited to the high-dose rats. Statistically increased mean absolute and relative adrenal weights of 15% and 17%, respectively, were observed at 1,500 mg/kg-day compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 8/12 treated rats, but not in controls. The hypertrophy was characterized as increased size and cytoplasmic eosinophilia of hepatocytes that were oriented around central veins, which at times extended into the midzonal region of the lobule. The severity was dose-related and ranged from minimal to moderate, and the authors suggested that the effect was similar to that observed with phenobarbital administration. Protein droplet nephropathy of the kidney was observed in 11/12 treated rats and 1/15 controls.

8.3.5 Three-Week Gavage Study In CD-1 Mice

CD-1 mice were administered methyl t-butyl ether via gavage five days per week for three weeks (Ward et al., 1994). This study was not available, but OEHHA (1999) and ATSDR (1996) indicated that no effects on body weight or unspecified reproductive parameters were observed at doses up to 1,000 mg/kg, and thus identified the NOAEL as 1,000 mg/kg (or 714 mg/kg-day).

8.4 Long-Term and Chronic Exposure Studies

Subchronic gavage exposures to methyl t-butyl ether were associated with increased mean absolute and relative kidney weights in male rats accompanied by hyaline droplet formation in the renal proximal tubules. In male and female rats administered methyl t-butyl ether at ≥100 mg/kg-day via gavage for 90 days, statistically increased mean blood urea nitrogen levels in of at least 15% compared to controls were observed. Chronic gavage exposure to methyl t-butyl ether was associated with an increase in Leydig cell tumors in male rats and leukemias/lymphomas (combined) in female rats.

8.4.1 Subchronic Studies

8.4.1.1 Four-Week Studies In Sprague-Dawley Rats

Ten Sprague-Dawley rats per sex and dose were administered methyl t-butyl ether (unspecified purity unspecified in water vehicle) via gavage at 0, 90, 440, or 1,750 mg/kg for five days per week for four weeks (Johnson et al., 1992; Klan et al., 1992). These doses were approximately equivalent to 0, 64, 314, or 1,250 mg/kg-day. Rats were housed individually, and food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Body weights were measured weekly. Hematology and clinical chemistry were conducted on all rats at study termination. Hematology included erythrocytes, platelets, leukocytes, differential leukocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, globulin, and albumin/globulin ratio. Clinical chemistry included glucose, creatine kinase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, sodium, pottasium, calcium, chloride, total protein and bilirubin, albumin, cholesterol and triglycerides. Adrenal, brain, ovary, testes, heart, kidney, liver, and spleen weights were measured, and relative organ-to-body-weight ratios were calculated. Gross necropsies were performed on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and included the adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eye, heart, ileum, jejunum, kidneys, liver, lung, mammary glands, muscle, nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, seminal vesicle, skin, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, and uterus. If effects were noted, the same organs were examined in the lower doses as well.

No non-gavage-related deaths occurred at any dose. At 64 mg/kg-day, transitory (<one hour after dosing) salivation was observed in several rats. Mean corpuscular hemoglobin was statistically increased in females by 4% compared to controls. Mean alkaline phosphatase was statistically increased in males by 15% compared to controls. Mean relative kidney weights were increased in females by 6% compared to controls.

At 314 mg/kg-day, transitory (<one hour after dosing) salivation was observed in all rats, and hypoactivity and/or ataxia was observed in several rats. Mean erythrocytes were statistically increased in males by 6% compared to controls. Mean relative kidney weights were statistically increased in males by 8% compared to controls. Hyaline droplet formation in the proximal convoluted tubules was observed in 7/10 males.

At 1,250 mg/kg-day, transitory (<one hour after dosing) salivation was observed in all rats, and hypoactivity and/or ataxia was observed in several rats. Mean corpuscular hemoglobin was statistically increased in females by 3% compared to controls. Mean total protein was statistically increased by 8% in females compared to controls, and cholesterol was statistically increased in males by 20% and females by 26% compared to controls. Mean relative kidney weights were increased in males by 13% and females by 17% compared to controls. Mean relative liver weights were increased in males by 8% and females by 12% compared to controls. Mean relative adrenal weights were increased in males by 19% compared to controls. Hyaline droplet formation in the proximal convoluted tubules was observed in 9/10 males. Various

effects in the stomach, including submucosal edema, subacute inflammation, epithelial hyperplasia, and ulceration were observed in up to 4/7 males and 5/10 females. The effects were largely confined to the forestomach.

The study authors concluded that the hyaline droplet formation in the proximal tubules in males was attributable to α-2μ-globulin nephropathy, which was not relevant to humans. Further, the stomach lesions were attributable to local irritation, which was not considered a direct result of systemic toxicity.

Fifteen male Sprague-Dawley rats per dose were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day methyl t-butyl ether (> 99.9% purity in corn oil) via gavage for 28 days (Williams and Borghoff, 2000; Williams et al., 2000). At study termination, the rats were sacrificed, body, adrenal, kidney, epididymides, testes, prostate, pituitary gland, seminal vesicle, and liver weights were determined, and histopathological examination of the liver, kidneys, testes, and adrenals was conducted.

There were no treatment-related deaths. Deaths attributed to gavage, which were confirmed at necropsy, were primarily limited to the high-dose rats. At 250 mg/kg-day, statistically increased mean relative kidney weights of 10% were observed compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 1/15 treated rats, but not in controls. The hypertrophy was characterized as increased size and cytoplasmic eosinophilia of hepatocytes that were oriented around central veins, which at times extended into the midzonal region of the lobule. The severity was dose-related and ranged from minimal to moderate, and the authors suggested that the effect was similar to that observed with phenobarbital administration. Protein droplet nephropathy of the kidney was observed in 12/15 treated rats, but not in controls.

At 500 mg/kg-day, statistically increased mean relative kidney weights of 9% were observed compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 10/15 treated rats, but not in controls. Protein droplet nephropathy of the kidney was observed in 15/15 treated rats, but not in controls.

At 1,000 mg/kg-day, statistically increased mean absolute and relative kidney weights of 10% and 16%, respectively, were observed compared to controls. Statistically increased mean relative liver weights of 10% were observed compared to controls. The increased relative liver weight was accompanied by minimal-to-moderate centrilobular hypertrophy in 11/13 treated rats, but not in controls. Protein droplet nephropathy of the kidney was observed in 12/13 treated rats, but not in controls.

At 1,500 mg/kg-day, mean body weight was reduced by 12% compared to controls. Statistically increased mean relative kidney weights of 18% were observed compared to controls. Statistically increased mean relative liver weights of 14% were observed compared to controls. Statistically increased mean relative testes weights of 15% were observed compared to controls. The increased relative liver weight was accompanied by minimal-to-moderate centrilobular hypertrophy in 11/11 treated rats, but not in controls. Increased mean relative kidney weights, accompanied by protein droplet nephropathy of the kidney, were observed in 10/11 treated rats, but not in controls.

8.4.1.2 Thirteen-Week Or Longer Studies In Sprague-Dawley Rats

Ten Sprague-Dawley rats per sex and dose were administered methyl t-butyl ether (≥ 99.95% purity in corn oil) via gavage at 0, 100, 300, 900, or 1,200 mg/kg-day for 90 days (Robinson et al., 1990). Rats were housed separately by sex and food, and water was available *ad libitum*. Mortality and clinical signs were monitored daily. Food consumption was measured once a week and water consumption was measured three times a week. Body weight was measured twice a week. Hematology and clinical chemistry were conducted on all rats at study termination. Hematology included hematocrit, hemoglobin, mean corpuscular volume, erythrocytes, leukocytes, differential leukocytes, and reticulocytes. Clinical chemistry included glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, cholesterol, calcium, and phosphorus. Brain, liver, spleen, lung, thymus, kidney, adrenal, heart, ovary, and testes weights were measured at study termination, and relative organ-to-body-weight ratios were calculated. Gross necropsies were conducted on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and included unspecified "major organs". If target histopathological organs were identified, these organs were also examined histologically in the remaining dose groups.

This study was not designed to meet current U.S. EPA (2007b) Health Effects Testing Guidelines, since hematology did not include a measure of clotting potential, and clinical chemistry did not include albumin, alkaline phosphatase, gamma glutamyl transferase, globulin, sorbitol dehydrogenase, bilirubin, protein, or serum chloride, magnesium, potassium, or sodium. Further, urinalysis was not conducted, and organs examined histologically were specified only as including "major organs".

At 100 mg/kg-day, one male died, but the cause of death was not specified. Diarrhea was observed in male and female rats. Water consumption (unspecified magnitude) was statistically increased in females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (15%) and females (20%).

At 300 mg/kg-day, one female died, but the cause of death was not specified. Diarrhea was observed in male and female rats. A statistical decrease in blood urea nitrogen was observed in males (20%) and females (33%). A statistical decrease in glucose (17%) and lactate dehydrogenase (62%) and an increase in cholesterol (11%) were observed in females. A statistical decrease in creatinine (15%) and an increase in aspartate aminotransferase (34%) were observed in males. Mean absolute (4%) and relative (4%) brain weights were statistically increased in males compared to controls. Mean relative kidney weights (10%) were statistically increased in females compared to controls.

At 900 mg/kg-day, two females and one male died, but the cause of death was not specified. Diarrhea was observed in male and female rats. Food consumption (unspecified magnitude) was statistically increased in females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (18%) and females (35%). A statistical decrease in mean glucose (13%) and lactate dehydrogenase (16%) and an increase in cholesterol (31%) were observed in females compared to controls. A statistical decrease in mean creatinine (26%) and an increase in cholesterol (22%) and lactate dehydrogenase (5%) were observed in males compared

to controls. Mean absolute (14%) and relative (15%) kidney weights were statistically increased in males compared to controls. Mean relative liver weights (13%) were statistically increased in males compared to controls. Mean relative heart (11%), liver (12%), kidney (13%), and thymus (33%) weights were statistically increased in females compared to controls.

At 1,200 mg/kg-day, four females and one male died, but the cause of death was not specified. Diarrhea and a profound but transient (<two hours) anesthetic effect were observed in male and female rats. Water consumption (unspecified magnitude) was statistically increased in males and females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (18%) and females (17%). A statistical decrease in mean glucose (24%) and lactate dehydrogenase (16%) and an increase in cholesterol (20%) were observed in females compared to controls. A statistical decrease in mean creatinine (19%) and an increase in aspartate aminotransferase (33%) were observed in males compared to controls. Terminal mean body weight was statistically reduced by 9% in males compared to controls. Mean absolute (18%) and relative (21%) kidney weights and mean absolute (9%) and relative (13%) lung weights were statistically increased in male rats compared to controls. Mean relative liver weights (12%) in males and kidney (12%) and adrenal (25%) weights in females were statistically increased in male rats compared to controls. According to the authors, microscopic findings included chronic nephropathy in both control and high-dose male rats. These changes, such as renal tubular degeneration, were more severe in treated rats than control rats. Renal tubules plugged with granular casts were found in 5/10 high-dose males, and 10/10 males exhibited slight increases in cytoplasmic hyaline droplets in proximal tubular epithelial cells. No further details regarding the renal changes were provided.

Ten male Sprague-Dawley rats per dose were administered 0, 200, 600, and 1,000 mg/kg methyl t-butyl ether (98.8% purity in soybean oil) by gavage for five days per week for 90 days (Zhou and Ye, 1999). These doses were equivalent to 0, 143, 428, or 857 mg/kg-day, respectively. Body weight and food and water consumption were measured weekly. Clinical chemistry was conducted at study termination and included aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, total protein, albumin, globulin, albumin/globulin ratio, blood urea nitrogen, and creatinine. Liver, kidney, testes, and lung weights were measured at study termination. Gross necropsies and histopathological examinations were conducted at study termination, and included the liver, kidney, testes, and lung. Liver sections were also examined under an electron microscope.

This study was not designed to meet current U.S. EPA (2007b) Health Effects Testing Guidelines, since only males were evaluated, hematology was not conducted, and clinical chemistry did not include alkaline phosphatase, gamma glutamyl transferase, glucose, sorbitol dehydrogenase, total bilirubin, total cholesterol, or serum electrolytes. Further, urinalysis was not conducted; spleen, heart, ovary, and brain weights were not measured; and histopathology included only the liver, kidney, testes, and lung.

At 143 mg/kg-day, mean absolute and relative liver weights were statistically increased by 12% and 14%, respectively, compared to controls. Lactate dehydrogenase was statistically decreased (32%) at the low, but not mid or high doses compared to controls. Aspartate aminotransferase was statistically increased by 31% compared to controls, but within historical control ranges.

Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. The study authors did, however, indicate that more severe changes were observed at higher doses.

At 428 mg/kg-day, mean absolute and relative liver weights were statistically increased by 18% and 15%, respectively, compared to controls. Mean relative kidney weight was statistically increased by 6% compared to controls, but no accompanying renal pathology was observed. Aspartate aminotransferase was statistically increased by 29% compared to controls, but within historical control ranges. Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. However, the study authors indicated that more severe changes were observed at higher doses.

At 857 mg/kg-day, mean absolute and relative liver weights were statistically increased by 21% and 22%, respectively, compared to controls. Mean absolute and relative kidney weights were statistically increased by 12% and 13%, respectively, compared to controls, but no accompanying renal pathology was observed. Aspartate aminotransferase was statistically increased by 27% compared to controls, but within historical control ranges. Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. However, the study authors indicated that more severe changes were observed at higher doses.

8.4.2 Chronic Studies

Sixty Sprague-Dawley rats per sex and dose were administered 0, 250, or 1,000 mg/kg methyl t-butyl ether (> 99% purity in extra virgin olive oil) by gavage four times a week for 104 weeks on a weekly schedule of two days dosing, one day without dosing, two days dosing, and two days without dosing (Belpoggi et al., 1995; 1997). These doses were approximately equivalent to daily doses of 0, 143, or 571 mg/kg-day. The animals were housed five per cage and kept under observation until natural death. Food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Food and water consumption and body weight were measured weekly for the first 13 weeks and twice monthly thereafter until 112 weeks. Thereafter, body weights were measured every eight weeks until death. Gross necropsies were performed on all rats after natural death. Histopathological examinations, which were performed on all rats after natural death, included the aorta, adrenals, bone, bone marrow, brain, bronchi, cecum, colon, diaphragm, duodenum, esophagus, eye, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (mediastinal, subcutaneous, mesenteric), mammary glands, muscles, nerve, ovaries, pancreas, pharynx, larynx, pituitary, prostate, salivary gland, seminal vesicle, subcutaneous tissue, skin, subcutaneous tissue, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, Zymbal gland, and gross lesions.

This study was not designed to meet current U.S. EPA (2007b) Health Effects Testing Guidelines, since the dosing occurred on a four-day per week schedule, with two days dosing, one day without dosing, two days dosing, and two days without dosing. Further, since no results for hematology, clinical chemistry, urinalysis, or organ weights were reported, it was presumed that these parameters were not examined. Histology did not include the aorta, bone, bone marrow, eye, mammary glands, muscles, nerve, seminal vesicle, or spinal cord. The tumor incidences reported in this study were reviewed by Belpoggi et al. (1998) after a re-evaluation of the histopathology slides.

At the low dose, survival at the end of the treatment period (104 weeks) was 35% in treated females compared to 48% in controls. Survival at the end of the treatment period (104 weeks) was 30% in low-dose males compared to 30% in controls. There was a statistical increase in lymphomas and leukemias combined (7/51) in female rats compared to controls (2/58). The individual incidence of lymphomas or leukemias was not indicated. The lymphatic tumors were accompanied by an increase in dysplastic proliferation of lymphoreticular tissue, which was characterized as hyperplastic lymphoid tissues at various sites, in which atypical lymphoid cells, usually lymphoimmunoblasts, isolated and/or aggregated in small clusters, were observed. An increased incidence of uterine sarcomas was observed in low-dose females, but not high-dose females, compared to controls.

At the high-dose, survival at the end of the treatment period (104 weeks) was 28% in treated females compared to 48% in controls. Survival at the end of the treatment period (104 weeks) was 42% in high-dose males compared to 30% in controls. There was a statistical increase in the incidence of testicular Leydig cell (interstitial cell) tumors in male rats compared to controls. The incidence was 3/26, 5/25, and 11/32 in control, low-, and high-dose males (based on the number of rats surviving at the occurrence of the first Leydig tumor, which was 96 weeks). In female rats, there was a dose-related statistical increase in lymphomas and leukemias combined (12/47) compared to controls (2/58), and an increase in dysplastic proliferation of lymphoreticular tissue. The study authors reported that the range of the lymphatic tumors in females in this study was within the historical control incidence for these tumors in female Sprague-Dawley rats from studies in their laboratory (below 10%).

The study authors reported that "no treatment-related non-oncological pathological changes were detected by gross inspection and histological examination", but the data were not provided.

8.5 Studies of Genotoxicity and Related End-Points

The genotoxicity data for methyl t-butyl ether have been critically reviewed by ECB (2002), OEHHA (1999), IPCS (1998), ECETOC (1997), and ATSDR (1996). The weight of evidence suggests that methyl t-butyl ether has some genotoxic potential. Methyl t-butyl ether has been tested in mutagenicity, chromosomal aberration, micronucleus, sister chromatid exchange, DNA damage and repair, and DNA strand break assays. Methyl t-butyl ether was not mutagenic in several *Salmonella* reverse mutation assays, although one assay was positive in TA102. Methyl t-butyl ether was also positive in a mouse lymphoma cell forward mutation assay, possibly due to the metabolism of methyl t-butyl ether to formaldehyde. Methyl t-butyl ether was negative in *in vivo* and *in vitro* chromosomal aberration assays, but equivocal results were observed in a sister

chromatid exchange assay *in vitro*. Methyl t-butyl ether was negative in *in vivo* and *in vitro* mouse micronucleus assays and unscheduled DNA synthesis assays, and an *in vivo* DNA repair assay, although methyl t-butyl ether was positive in a DNA strand break assay in rat lymphocytes *in vivo* and human lymphocytes *in vitro*. DNA adduct formation was observed in mice given a single gavage dose of methyl t-butyl ether.

8.5.1 Mutagenicity Assays

Methyl t-butyl ether was not mutagenic in *Salmonella* reverse mutation assays and tissue culture mutation assays, and was negative in a *Drosophila* sex-linked recessive assay (OEHHA, 1999). Methyl t-butyl ether was positive in one *Salmonella* reverse mutation assay in strain TA102 and negative in eight other *Salmonella* reverse mutation assays, two of which included TA102 (ECB, 2002). In a study by Williams-Hill et al. (1999), methyl t-butyl ether (99.9% purity) was weakly and moderately positive in TA102 without and with metabolic activation, respectively. Addition of formaldehyde dehydrogenase reduced the mutagenic potential, suggesting that the formaldehyde metabolite was partially responsible for the positive result. A well-conducted study by McGregor et al. (2005) failed to replicate the positive result for methyl t-butyl ether in *Salmonella* TA102.

According to ECB (2002), methyl t-butyl ether was evaluated in an *in vitro* forward mutation assay in mouse L5178Y TK+/- lymphocytes at concentrations of 0.39 to 6.25 µl/ml with and without metabolic activation with the liver S9 fraction from Fisher-344 rats (ARCO, 1980; Mackerer et al., 1996). After a two- to three-day recovery and expression period, lymphocytes were plated and incubated with methyl t-butyl ether (96% or 99% purity) for 10 days. The total number of resistant colonies was counted, and the ratio to cells growing in non-selective medium was determined and characterized as the mutant frequency. Five parallel assays were conducted. Methyl t-butyl ether showed a statistically and dose-dependently increased mutation frequency in the presence of metabolic activation when compared to controls. Mackerer et al. (1996) then investigated the possible role of formaldehyde in the mutagenic events. The authors exposed mouse lymphoma cells to concentrations of methyl t-butyl ether from 1 to 4 µl/ml for three hours and added formaldehyde dehydrogenase with its co-factor NAD+, both of which convert formaldehyde to non-mutagenic formic acid, thereby eliminating possible mutagenicity resulting from formaldehyde. The results showed that the mutation frequency did not increase when formaldehyde dehydrogenase and its coenzyme were present, while there was a five-fold increase in its absence.

ECB (2002) reported that methyl t-butyl ether was evaluated in two separate studies for the ability to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster V79 cells with and without metabolic activation. In the studies by Life Science Research (1989) and Cinelli et al. (1992), no statistical increase in mutation frequency was observed compared to controls.

8.5.2 Assays of Chromosomal Damage

Methyl t-butyl ether was negative in *in vivo* and *in vitro* chromosomal aberration assays (ECB, 2002; OEHHA, 1999), and in an *in vitro* sister chromatid exchange assay (OEHHA, 1999). ECB

(2002) considered the results from the *in vitro* sister chromatid exchange assay to be equivocal, since there was a significant increase of sister chromatid exchange frequency in one of the two replicates at 1µl/ml methyl t-butyl ether (99% purity). However, because there was only a small increase of sister chromatid exchange induction with the positive control, the test was repeated, and repeat tests failed to confirm the positive result (Litton Bionetics, 1980). Further, a concurrent assay using methyl t-butyl ether of 95% purity failed to demonstrated a statistical increase in sister chromatid exchange frequency compared to controls.

8.5.3 Other Assays of Genetic Damage

Methyl t-butyl ether was negative in *in vivo* and *in vitro* mouse micronucleus assays (ECB, 2002; OEHHA, 1999), *in vivo* and *in vitro* unscheduled DNA synthesis assays, and an *in vivo* DNA repair assay (OEHHA, 1999).

ECB (2002) reported that methyl t-butyl ether was positive in one and negative in two unscheduled DNA synthesis assays in primary rat hepatocytes and *in vitro*. In the positive study, methyl t-butyl ether was evaluated for unscheduled DNA synthesis in primary rat hepatocytes isolated from two male Sprague-Dawley rats (Zhou et al., 2000). Hepatocytes were incubated with 0, 200, 600 or 1,000 ug/ml methyl t-butyl ether (98.8% in dimethylsulfoxide) and 5 µCi/mL [^{3}H]-methylthymidine for three hours at 37°C. Radioactivity was measured with a liquid scintillation spectrometer. Concurrent negative, vehicle (dimethylsulfoxide) and positive controls (mechlorethamine) were included. At 0, 0.2, 0.6 or 1 mg/mL, the radioactivity (counts per minute) was 712, 777, 1,311, and 1,437 CPM, respectively. The highest dose was statistically significant when compared to vehicle controls.

Methyl t-butyl ether was evaluated in the single cell gel electrophoresis assay (comet assay) *in vitro* for the ability to induce DNA strand breaks in human lymphocytes (Chen et al., 2007). The assay was conducted under neutral and alkaline pH conditions. Lymphocytes isolated from the blood of one healthy female donor were incubated with methyl t-butyl ether (unspecified purity in dimethylsulfoxide) at 0, 0.05, 0.1, or 0.2 mM for one hour. After cell viability was determined to be >95%, one hundred comets on each of triplicate slides were scored visually according to the relative intensity of the tail. An intensity score from class 0 (undamaged) to class 4 (severely damaged) was assigned to each cell. Thus, the total score for the 100 comets could range from 0 to 400. The extent of DNA damage was analyzed and scored by the same experienced person. Solvent and positive controls were included. The mean DNA damage score was statistically increased at all doses compared to controls (18%, 24%, and 26%, respectively, under alkaline conditions and 47%, 65%, and 78%, respectively, under neutral conditions).

Methyl t-butyl ether was evaluated in the alkaline single cell gel electrophoresis assay (comet assay) *in vivo* for the ability to induce DNA strand breaks in rat lymphocytes (Lee et al., 1998). Nine male Sprague-Dawley rats per dose were administered methyl t-butyl ether via gavage at 0, 40, 400, or 800 mg/kg for 28 days. Lymphocytes isolated from trunk blood were analyzed for DNA strand breaks and scored for apoptosis frequency. Cytotoxicity (or viability) was determined by the trypan blue exclusion method. Viability was greater than 90% in all groups. Measures of DNA-strand breakage, such as tail length and tail moment, were significantly

increased at 800 mg/kg compared to controls. As this publication was an abstract, and a full publication was not identified, no further details were available.

The potential DNA adduct formation has been examined in male Kunming mice given ^{14}C-methyl t-butyl ether (>95% purity) at up to 1.9 mg/kg (Yuan et al., 2007) or 6.18 mg/kg (Du et al., 2005) via gavage. DNA was extracted six hours post-dosing from lung, liver and kidney samples. DNA adducts were detected in a dose-related manner in the kidney and lung at 1.33 mg/kg and above. The methyl group of methyl t-butyl ether was shown to be the predominant binding moiety in liver, while the methyl group and the tert-butyl group gave comparable contributions to adduct formation in lung and kidney (Yuan et al., 2007).

Iavicoli et al. (2002) investigated the ability of methyl t-butyl ether to induce cytotoxicity, transformation, or apoptosis of rat fibroblasts *in vitro*. In the cell proliferation and cytoxicity assay, rat-1 normal rat fibroblasts were incubated with methyl t-butyl ether (99.8% purity) at 8.4 x 10^{-6} to 8.4 mM for 48 hours. After rinsing, the cultures were then exposed to 3-(4,5-)-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for two hours. The amount of the colored formazan derivative that was formed was measured to determine cytotoxicity. Cell viability after a two-day incubation was also determined. DNA content was measured using flow-assisted cell sorting. Cell cycle analysis, to measure apoptosis, was conducted after a 48-hour exposure to methyl t-butyl ether at the IC_{50} or 1/10 of the IC_{50} (0.84 or 0.084 mM, respectively). Twenty-four hour cell transformation assays were also conducted in C3H/10$T_{1/2}$ Cl 8 mouse embryo fibroblasts at methyl t-butyl ether concentrations of 0.336 and 0.672 mM, with cigarette tobacco smoke serving as a positive control. These doses were selected since they did not cause cell loss after 24 hours. All assays were performed in triplicate.

The 50% inhibitory concentration (IC_{50}) of methyl t-butyl ether on growth of rat fibroblasts was determined to be 0.84 mM. Cell viability at the IC_{50} or 1/10 of the IC_{50} (0.84 or 0.084 mM, respectively) after 24 and 48 hours was statistically and dose- and time-dependently reduced compared to controls. Methyl t-butyl ether also caused a dose-dependent reduction in the number of cells in the G2/M phase of the cell cycle and an increase in the percentage of cells in the S-phase, indicating increased apoptosis. At 0.336 and 0.672 mM methyl t-butyl ether, a statistical increase in the number of transformed cell loci in mouse embryo fibroblasts of 2.1 and 2.5 times the control, respectively, was observed.

8.6 Reproductive and Developmental Toxicity Studies

No *in vivo* oral two-generation reproduction or developmental studies were identified for methyl t-butyl ether. In an attempt to characterize a possible mode of action for the Leydig cell tumors observed in male rats after chronic gavage dosing, reproductive effects in male rats have recently been investigated in non-standardized reproduction studies. A single gavage dose of methyl t-butyl ether at approximately 500 mg/kg-day resulted in reduced circulating testosterone in male rats during the hours immediately following dosing. In male rats treated with 1,200 mg/kg-day methyl t-butyl ether for 14 days, decreased mean testosterone and luteinizing hormone and increased estradiol were observed, along with decreased testicular microsomal aromatase activity. Repeated exposure to 800 mg/kg-day methyl t-butyl ether via gavage in male rats was associated with statistical reductions in circulating testosterone after 28 days. High doses (> 50

mM) of methyl t-butyl ether were also found to reduce basal and human Chorionic Gonadotropin (hCG)-stimulated testosterone production in Leydig cells *in vitro*.

One- and two-generation inhalation reproductive studies in rats and four inhalation developmental studies in rats, mice, and rabbits are available for methyl t-butyl ether. These studies have been reviewed by ECB (2002), OEHHA (1999), IPCS (1998), ECETOC (1997), and ATSDR (1996). Specific reproductive effects were not observed in rats at concentrations up to 28,800 mg/m^3. Methyl t-butyl ether did not induce developmental effects at concentrations below those that were maternally toxic. Decreases in uterine weight and increases in estrogen metabolism in mice have been observed at 28,800 mg/m^3 (IPCS, 1998).

Since the reproductive and developmental studies for methyl t-butyl ether have been extensively reviewed by several other regulatory agencies, this section includes only the oral reproductive and developmental studies for methyl t-butyl ether, including those published since the various regulatory reviews. However, none of the oral studies were standardized two-generation reproduction or developmental studies.

8.6.1 Reproduction Studies

No oral one- or two-generation reproduction studies were identified for methyl t-butyl ether, but effects on the reproductive organs have been investigated following oral exposure to methyl t-butyl ether in non-standardized reproduction studies.

Potential testicular toxicity associated with methyl t-butyl ether was assessed in five male CD-1 mice per dose that received gavage doses of methyl t-butyl ether (unspecified purity in canola oil) on Days 1, 3, and 5 at 0, 400, 1,000 or 2,000 mg/kg (Billitti et al., 2005). Testosterone levels were measured on Day 6 fecal samples collected from all mice. Thereafter, mice were injected with human chorionic gonadotrophin to stimulate maximum testosterone production and fecal samples were collected after one day. Body weight and serum testosterone were measured and histological examination of the testes was included at study termination. Two high-dose mice died as a result of dosing error. All examined parameters in the treated mice that survived were comparable and/or not statistically different compared to controls.

Eight female B6C3F$_1$ mice per dose were given methyl t-butyl ether (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Twenty-four hours after the last dose, the mice were sacrificed and hepatocytes were isolated for measurement of estrogen metabolism *in vitro*, which was expressed as the amount (nM) of 17-β–estradiol metabolized/ mg protein/ minute. Methyl t-butyl ether induced a two-fold statistical increase in the rate of estrogen metabolism *in vitro* compared to controls.

Six to eleven female CD-1 mice per dose were administered methyl t-butyl ether via gavage at 0, 600, or 1,500 mg/kg-day for five days either with or without subcutaneous administration of 1 ug estradiol on Days 3-5 (Okahara et al., 1998). The authors reported that methyl t-butyl ether had some mild, but in some cases, seemingly opposite, activity under these conditions, but no further details were provided. At 1,500 mg/kg-day, delayed vaginal opening by Postnatal Day 26 was

observed in half of the treated females. Mean relative uterine weights were statistically increased in the methyl t-butyl ether/estradiol group compared to the estradiol alone control group, but the dose level or magnitude was not specified. According to the authors, no clear or consistent effect was observed in uterine peroxidase activity or in ovarian, liver, or kidney weights compared to controls. No further details were available in this abstract, and a full publication was not located.

Twelve male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0, 1,000, or 1,500 mg/kg every other day for a total of 14 doses over 28 days (de Peyster et al., 2003). The 1,000 mg/kg dose was selected since it was the highest dose in the Belpoggi et al. (1995) chronic gavage study, and since this dose induced a statistical increase in Leydig cell tumors in male rats compared to controls. The 1,500 mg/kg dose was chosen since it was approximately the highest dose from a 90-day gavage study for methyl t-butyl ether by Robinson et al. (1990). The experiment originally included an untreated and a vehicle-treated control group, but the results were ultimately combined into one control group. Due to excess weight loss and one death, the 1,000 and 1,500 mg/kg doses were reduced to 500 and 750 mg/kg, respectively, starting on Day 13. The terminal doses were approximately equivalent to 0, 357, or 536 mg/kg-day. This study was conducted to investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to methyl t-butyl ether in a study by Belpoggi et al. (1995). It has been suggested that increased hepatic metabolism through P450 enzymes results in increased steroid catabolism, resulting in reduced testosterone circulation.

Testosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (cardiac puncture). If the serum sample volume was sufficient, terminal corticosterone was also measured to determine whether the Leydig tumors were induced through an increased stimulation of testicular glucocorticoid receptors, which can impair testosterone production. Liver, kidney, testes, seminal vesicles, and epididymides weights were measured, and mean organ-to-body weight ratios were calculated. Total protein and total P450 were measured from isolated liver microsomes.

At study termination, mean body weight gain was 8, 3, 1, and 0% in the negative control, vehicle control, 357 mg/kg-day, and 536 mg/kg-day groups, respectively. The Day 1 testosterone concentration in rats administered 537 mg/kg-day methyl t-butyl ether was statistically reduced by approximately 70% compared to pooled controls (vehicle and negative, n=4 only). The Day 14 and 28 testosterone concentrations in treated rats were not statistically different compared to controls. At study termination, mean absolute liver weight and total microsomal protein in treated rats were comparable to controls, but mean liver P450 content (mmol/mg protein and nmol/g liver weight) was slightly, but statistically, increased in rats administered 537 mg/kg-day compared to controls. There was a 24% increase in mmol/mg P450 protein and a 35% increase in nmol P450/g liver weight compared to pooled controls. Mean corticosterone levels on Day 1, 14, and 28 were not statistically different compared to pooled controls, but the sample size was only about 4-5 rats per dose. The authors concluded that high gavage doses of methyl t-butyl ether result in reduced circulating testosterone in rats during the hours immediately following dosing (4-5 hours). However, the increase in hepatic P450 content did not result in reduced

circulating testosterone, as originally hypothesized by the study authors, but the authors could not rule out other hormonal or metabolic compensatory mechanisms.

Twelve male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0, 40, 400, or 800 mg/kg-day for 28 days (de Peyster et al., 2003; Day et al., 1998). This study was conducted to further investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to methyl t-butyl ether in a study by Belpoggi et al. (1995). Luteinizing hormone, prolactin, testosterone, and corticosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (trunk). Liver, pituitary, testes, epididymides, thyroid, adrenal, prostate, and brain weights were measured, and mean organ-to-body and brain weight ratios were calculated.

At 40 mg/kg-day, mean plasma corticosterone was statistically reduced by 28% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 400 mg/kg-day, terminal mean body weight was statistically reduced by 7% compared to controls. Mean plasma corticosterone was statistically reduced by 42% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination. Mean pituitary weight was statistically reduced by 23% compared to controls.

At 800 mg/kg-day, terminal mean body weight was statistically reduced by 13% compared to controls. Mean plasma corticosterone was statistically reduced by 43% compared to controls on Day 14. At study termination, mean plasma testosterone was statistically reduced by 35% and mean plasma corticosterone was statistically reduced by 44% compared to controls. The mean adrenal-to-body-weight ratio was statistically reduced by 20% compared to controls. The mean thyroid-to-body-weight ratio was statistically reduced by 29% compared to controls.

Six male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0 or 800 mg/kg-day for five days (de Peyster et al., 2003). This study was conducted to further investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to methyl t-butyl ether in a study by Belpoggi et al. (1995). The effect of castration on the hypothalamic-pituitary axis was investigated using testosterone implants in phosphate buffered saline (PBS) and four experimental groups of male rats. The four groups consisted of sham implant (PBS) and 800 mg/kg-day methyl t-butyl ether via gavage, sham implant (PBS) and corn oil vehicle gavage, testosterone implant and 800 mg/kg-day methyl t-butyl ether via gavage, and testosterone implant and corn oil vehicle gavage. The amount of testosterone in each implant was intended to result in average circulating testosterone as in normal non-castrated rats. Lutenizing hormone, prolactin, and testosterone concentrations from the tail vein were measured four hours after the initial dose (Day 1) and two hours after the final dose (Day 5). Terminal prostate and seminal vesicle weights were measured. The experiment was repeated with a younger set of animals, reportedly to reduce the amount of body weight variation, since each testosterone implant contained a standard amount of testosterone.

In the first experiment, the authors found that circulating testosterone was higher and lutenizing hormone was lower in rats with testosterone implants compared to controls, but the differences

were not statistically significant. Since each testosterone implant contained a standard amount of testosterone, the authors suggested that the results were confounded by the difference in body weights between the rats after the 3-day recovery period from the surgical implant, even though prior to surgery, the rats were of comparable body weights. Thus, the experiment was repeated with a younger set of animals, but the results of the first experiment could not be duplicated and may have been confounded by a small sample size, since one control rat gained a large amount of body weight. Recognizing confounding factors, the authors concluded that there was no clear evidence of an effect on the hypothalamic-pituitary axis in either experiment.

Ten male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0 or 1,200 mg/kg-day for 14 days (de Peyster et al., 2003). This dose was determined in previous experiments to lower circulating testosterone levels without affecting body weight. Terminal plasma estradiol, luteinizing hormone, and testosterone concentrations were measured from trunk blood samples. Testes and accessory sex organs (unspecified) weights were measured. Total protein content in testicular microsomes was determined, and testicular microsomal aromatase activity was also measured.

In rats treated with 1,200 mg/kg-day methyl t-butyl ether, a statistical decrease in mean testosterone and luteinizing hormone of 51% and 10%, respectively, was observed compared to controls, and a statistical increase in mean estradiol of 26% was observed compared to controls. Testicular microsomal aromatase activity was decreased by 55% compared to controls.

Williams and Borghoff (2000) and Williams et al. (2000) investigated the hypothesis that methyl t-butyl ether-induced decrease in serum testosterone levels in male rats may be due in part to the ability of methyl t-butyl ether to induce the metabolism of endogenous testosterone and, hence, enhance its clearance. Male Sprague-Dawley rats were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day methyl t-butyl ether (> 99.9% purity in corn oil) via gavage for 15 or 28 days. Rats were sacrificed one hour following the last dose, and serum and interstitial fluid testosterone, and serum dihydrotestosterone, 17-β-estradiol, prolactin, triiodothyronine (T3), thyroxin (T4), thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone levels were measured. Histopathology of the testes was performed in all rats.

After 15 days at 1,500 mg/kg-day, interstitial fluid and serum testosterone levels (approximately 60% each, estimated from graph) and serum prolactin levels (56%) were statistically decreased compared to controls.

After 28 days at 1,000 mg/kg-day, serum triiodothyronine (T3) was statistically decreased by 19% compared to controls.

After 28 days at 1,500 mg/kg-day, serum triiodothyronine (T3; 19%), luteinizing hormone (approximately 20%, estimated from graph), and dihydrotestosterone (45%) were statistically decreased compared to controls.

No testicular lesions were observed at any dose level. The authors concluded that methyl t-butyl ether causes mild perturbations in T3 and prolactin; however, the short-term (15-day), but not longer-term (28-day), decrease in testosterone and the mild increase in luteinizing hormone

levels did not fit the pattern caused by known Leydig cell tumorigens, since larger increases in luteinizing hormone have been caused by chemicals known to cause Leydig cell tumors.

Ten CD-1 mice per sex and dose were given 0, 1, 10, 100 or 1,000 mg/kg methyl t-butyl ether (purity unspecified in corn oil) by gavage for five days per week for three weeks (Ward et al., 1994). As this study was not available, this summary was based on IPCS (1998). These doses were approximately equivalent to 0, 0.7, 7, 71, or 714 mg/kg-day. At study termination, the mice were sacrificed and one testis from each male and both ovaries from each female were sectioned for cytological evaluation. In males, sperm number, Sertoli cells, spermatogonia, spermatocytes, and capped spermatids were evaluated. In females, oocyte quality was assessed. There were no effects of methyl t-butyl ether on any of the cell types examined, but no further details were provided. OEHHA (1999) and ATSDR (1996) indicated that the reproductive NOAEL for this study was 1,000 mg/kg-day, but no further details were available. It should be noted that OEHHA (1999) and ATSDR (1996) likely did not adjust for the less than daily dosing regimen, and likely should have indicated the reproductive NOAEL as 714 mg/kg-day.

The effect of methyl t-butyl ether on the testosterone production of Leydig cells in culture was examined *in vitro* by de Peyster et al. (2003). Leydig cells were isolated from adult male Sprague-Dawley rats and incubated for three hours with 0, 50, or 100 mM methyl t-butyl ether (> 99.8% purity) or t-butanol, a major metabolite of methyl t-butyl ether. The same concentrations were also tested with human Chorionic Gonadotropin (hCG), added to stimulate testosterone production. Cell viability at the tested concentrations was at least 85%. Testosterone production after the three-hour exposure was measured by radioimmunoassay. Aminoglutethimide was used as a positive control, and the experiment was conducted in triplicate.

A statistical reduction in basal testosterone production of 56% and 76%, compared to controls, was observed at 50 and 100 mM methyl t-butyl ether, respectively. A statistical reduction in human Chorionic Gonadotropin-stimulated testosterone production of 51% and 60%, compared to controls, was observed at 50 and 100 mM methyl t-butyl ether, respectively. T-butanol induced a statistical reduction in basal testosterone production of 72% and 66% at 50 mM and 100 mM compared to controls, respectively. T-butanol induced a statistical reduction in human Chorionic Gonadotropin-stimulated testosterone production of 73% and 83% at 50 mM and 100 mM compared to controls, respectively. The positive control, aminoglutethimide (5 mM) induced a statistical reduction of basal and human Chorionic Gonadotropin-stimulated testosterone production of 80% and 75% compared to controls, respectively.

In a 14- and 90-day systemic gavage study in Sprague-Dawley rats by Robinson et al. (1990), effects on ovary weight and histology and testes weight and histology were examined, and no effects were reported.

8.6.2 Developmental Toxicity Studies

No oral developmental studies were identified for methyl t-butyl ether.

8.7 Studies of Immunological and Neurological Effects

No standardized immunological or neurological assays were identified for methyl t-butyl ether, but some immunological or neurological effects have been reported in systemic studies for methyl t-butyl ether. Reported immunological effects were limited to reduced circulating corticosterone levels and thyroid weights in rats after short-term gavage exposures. Reported neurological effects were limited to transitory salivation after a single gavage dose of 90 mg/kg-day and higher and transitory hypoactivity and/or ataxia at higher doses in rats.

8.7.1 Immunological Effects

Twelve male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0, 1,000, or 1,500 mg/kg every other day for a total of 14 doses over 28 days (de Peyster et al., 2003). After an adjustment of doses due to excess weight loss, the terminal doses were approximately equivalent to 0, 357, or 536 mg/kg-day. Terminal corticosterone was measured on Day 1, 14, and 28. Mean corticosterone levels on Day 1, 14, and 28 were not statistically different compared to controls, but the sample size was only about 4-5 rats per dose, due to other analyses concurrently requiring blood volume.

Twelve male Sprague-Dawley rats per dose were administered methyl t-butyl ether (> 99.8% purity in corn oil) via gavage at 0, 40, 400, or 800 mg/kg-day for 28 days (de Peyster et al., 2003). Corticosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (trunk). Thyroid weights were measured, and mean organ-to-body and brain weight ratios were calculated.

At 40 mg/kg-day, mean plasma corticosterone was statistically reduced by 28% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 400 mg/kg-day, mean plasma corticosterone was statistically reduced by 42% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 800 mg/kg-day, mean plasma corticosterone was statistically reduced by 43% compared to controls on Day 14. At study termination, mean plasma corticosterone was statistically reduced by 44% compared to controls. The mean thyroid-to-body- weight ratio was statistically reduced by 29% compared to controls.

In a 14-day and 90-day systemic gavage study in Sprague-Dawley rats by Robinson et al. (1990), effects on spleen and thymus weight and histology were examined, and no effects were reported. Although some statistical reductions in monocyte differential counts were observed, the effect was not dose- or duration-related and did not occur in both sexes.

In a 28-day gavage study by Lee et al. (1998), methyl t-butyl ether (unspecified purity in corn oil) was administered to male Sprague-Dawley rats at 0, 40, 400, or 800 mg/kg-day via gavage. At 800 mg/kg-day, high corticosterone levels were observed, but the magnitude and statistical

significance were not specified. Limited details were available in this published abstract and a full publication was not located.

8.7.2 Neurological Effects

In rats, reported neurological effects were limited to transitory salivation reported after a single gavage dose of 90 mg/kg-day and higher and transitory hypoactivity and/or ataxia at higher doses (Johnson et al., 1992).

Martin et al. (2002) studied the effect of 200 and 400 mM methyl t-butyl ether (unspecified purity) on binding at the gamma-aminobutyric acid receptor site in cerebral cortex membrane preparations isolated from male Sprague-Dawley rats. The gamma-aminobutyric acid receptor was probed using the ^{3}H-t-butylbicycloorthobenzoate, which binds to the convulsant recognition site of the receptor. The experiment was conducted in triplicate.

The 50% inhibitory concentration (IC_{50}) of methyl t-butyl ether and its metabolite, t-butanol, on the binding of ^{3}H-t-butylbicycloorthobenzoate at the gamma-aminobutyric acid(A) receptor site was 120 and 69 mM, respectively. In additional saturation binding assays, 200 and 400 mM methyl t-butyl ether statistically reduced apparent density of convulsant binding, or B_{max}, to 36 and 17% of the control value, respectively. The study authors suggested that their results indicate that direct effects on the gamma-aminobutyric acid(A) receptor site by methyl t-butyl ether or its metabolite t-butanol could explain some of the neurotoxicological or neurobehavioral effects observed after methyl t-butyl ether exposures in humans and laboratory animals.

9.0 RISK CHARACTERIZATION

9.1 Hazard Identification

Oral LOAEL and NOAEL values from the animal studies reviewed are shown in Table 2.

9.1.1 Evaluation of Major Non-Cancer Effects and Mode of Action

9.1.1.1 Major Non-Cancer Effects

The scientific literature for methyl t-butyl ether in humans and laboratory animals has been reviewed extensively by several national and international regulatory agencies, including the European Chemicals Bureau (ECB, 2002), Office of Environmental Health Hazard Assessment of the California EPA (OEHHA, 1999), the International Agency for Research on Cancer (IARC, 1999), the International Programme on Chemical Safety of the World Health Organization (IPCS, 1998), the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1992). Thus, this risk assessment to determine drinking water action levels for methyl t-butyl ether focuses mainly on the oral exposure studies included in these reviews or that have been published since these reviews.

Table 2. Summary of non-cancer LOAEL and NOAEL values from repeated-dose oral studies with methyl t-butyl ether

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	LOAEL mg/kg-day	Non-Cancer Biological Effect(s)	Reference
Two-Week (Sprague-Dawley Rat)	Gavage	None (Females) 357 (Males)	357 (Females) 714 (Males)	↓ mean absolute and relative lung weights in male and female rats and altered clinical parameters in male rats. Limited endpoints evaluated.	Robinson et al., 1990
Two-Week (Sprague-Dawley Rat)	Gavage	800 (Males only)	None (Males only)	No effect on hepatic clinical chemistry or peroxisomal proliferation. Limited endpoints evaluated.	DePeyster et al., 2003
Three-Week (CD-1 Mouse)	Gavage	714[a]	None	No effects on body weight or reproductive parameters (sperm number, Sertoli cells, spermatogonia, spermatocytes, and capped spermatids in males and oocyte quality in females). Limited endpoints evaluated.	Ward et al., 1994[b]; 1995[b]
Four-Week (Sprague-Dawley Rat)	Gavage	250 (Males only)	500 (Males only)	↑ mean relative liver weight and minimal-to-moderate centrilobular hypertrophy. ↑ mean relative kidney weight and protein droplet nephropathy in renal tubules. Limited endpoints evaluated.	Williams and Borghoff, 2000; Williams et al., 2000
Four-Week (Sprague-Dawley Rat)	Gavage	314 (Males and Females)[a]	1,250 (Males and Females)[a]	↑ mean cholesterol and relative liver weight. Gastric inflammation, edema, hyperplasia, and ulcers. ↑ mean relative kidney weight and hyaline droplet formation in renal tubules of males. Limited endpoints evaluated.	Johnson et al., 1992; Klan et al., 1992
Four-Week (Sprague-Dawley Rat)	Gavage	357 (Males only)	536 (Males only)	↓ circulating testosterone concentration immediately following dosing; ↑ mean liver P450 content. Limited endpoints evaluated.	DePeyster et al., 2003
Four-Week (Sprague-Dawley Rat)	Gavage	400 (Males only)	800 (Males only)	↓ body weight and ↓ plasma testosterone and corticosterone. Limited endpoints evaluated.	DePeyster et al., 2003; Day et al., 1998
Four-Week (Sprague-Dawley Rat)	Gavage	None (Males only)	1,200 (Males only)	↑ mean relative liver weight, ↓ mean testosterone and luteinizing hormone, ↑ mean estradiol, ↓ hepatic and testicular microsomal aromatase activity. Limited endpoints evaluated.	DePeyster et al., 2003
13-Week (Sprague-Dawley Rat)	Gavage	None (Males only)	143 (Males only)[a]	↑ liver weights and aspartate aminotransferase, hepatic nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration. Limited endpoints evaluated.	Zhou and Ye, 1999
13-Week (Sprague-Dawley Rat)	Gavage	None (Males and Females)	100 (Males and Females)	↓ blood urea nitrogen in males and females. Limited endpoints evaluated.	Robinson et al., 1990

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	LOAEL mg/kg-day	Non-Cancer Biological Effect(s)	Reference
104-Week (Sprague-Dawley Rat)	Gavage	Not determined		Although study authors reported "no treatment-related nononcological pathological changes were detected by gross inspection and histological examination," data were not provided. Limited endpoints evaluated (no hematology, clinical chemistry, or urinalysis).	Belpoggi et al., 1995; 1997; 1998

[a] Doses were adjusted to account for a less than 7-day dosing regimen.
[b] Study not available, and thus as cited in OEHHA (1999) and ATSDR (1996).

No evidence of hepatic peroxisome proliferation was observed in male rats administered methyl t-butyl ether via gavage at 800 mg/kg-day for 14 days (de Peyster et al., 2003), but increased mean relative liver weight and minimal-to-moderate centrilobular hypertrophy were observed in male rats administered methyl t-butyl ether via gavage at 1,000 mg/kg-day and above for 28 days (Williams and Borghoff, 2000; Williams et al., 2000). In male and female rats administered high doses of methyl t-butyl ether (1,250 mg/kg-day) via gavage for 28 days, statistically increased cholesterol levels of at least 20% compared to controls were observed (Johnson et al., 1992).

Short-term and subchronic gavage exposures to methyl t-butyl ether were associated with increased mean absolute and relative kidney weights in male rats accompanied by hyaline droplet formation in the renal proximal tubules. In male and female rats administered methyl t-butyl ether at 100 mg/kg-day and above via gavage for 13 weeks, statistically increased mean blood urea nitrogen levels of at least 15% compared to controls were observed (Robinson et al., 1990). Statistically increased liver weights and aspartate aminotransferase, along with hepatic nuclear condensation, fat droplets, lysosome appearance in hepatocytes, and smooth endoplasmic reticulum disintegration were observed after subchronic gavage doses of 143 mg/kg-day and higher (Zhou and Ye, 1999). After chronic gavage exposure to methyl t-butyl ether at doses up to 571 mg/kg-day, Belpoggi et al. (1995) reported that "no treatment-related nononcological pathological changes were detected by gross inspection and histological examination". However, the data were not provided. Thus, increases in liver weight, aspartate aminotransferase, blood urea nitrogen, and cholesterol and the centrilobular hepatocyte hypertrophy observed in rats after short-term and subchronic oral exposures to methyl t-butyl ether could not be critically assessed following chronic oral exposures.

No *in vivo* oral two-generation reproduction or developmental studies were identified for methyl t-butyl ether. Reproductive effects in male rats have been investigated in non-standardized reproduction studies. A single gavage dose of methyl t-butyl ether at approximately 500 mg/kg-day resulted in reduced circulating testosterone in male rats during the hours immediately following dosing (de Peyster et al., 2003). In male rats treated with 1,200 mg/kg-day methyl t-butyl ether for 14 days, decreased mean testosterone and luteinizing hormone and increased estradiol were observed, along with decreased testicular microsomal aromatase activity (de Peyster et al., 2003). Repeated exposure to 800 mg/kg-day methyl t-butyl ether via gavage in male rats was associated with statistical reductions in circulating testosterone after 28 days (de Peyster et al., 2003). High doses (> 50 mM) of methyl t-butyl ether were also found to reduce basal and human Chorionic Gonadotropin (hCG)-stimulated testosterone production in Leydig cells *in vitro*.

No standardized immunological or neurological assays were identified for methyl t-butyl ether, but some immunological or neurological effects have been reported in systemic studies for methyl t-butyl ether. Reported immunological effects were limited to reduced circulating corticosterone levels and thyroid weights in rats after short-term gavage exposures. Studies for other chemicals have demonstrated that the effects of a chemical stressor on selected immunological parameters can be predicted on the basis of the area under the corticosterone concentration versus time curve (Pruett et al., 2003). Reported neurological effects were limited

to transitory salivation after a single gavage dose of 90 mg/kg-day and higher and transitory hypoactivity and/or ataxia at higher doses in rats.

9.1.1.2 Mode of Action (Non-Cancer Effects)

In vivo and *in vitro* assays by de Peyster et al. (2003) demonstrated that single and repeated gavage exposures to methyl t-butyl ether can reduce circulating testosterone levels. Williams and Borghoff (2000) demonstrated that methyl t-butyl ether induced mild increases in testosterone hydroxylase enzymes. Further, increases in UDP-glucuronosyltransferase observed were consistent with the centrilobular hypertrophy observed in rodents after repeated methyl t-butyl ether exposures. Collectively, the study authors suggested that the decrease in serum testosterone observed following methyl t-butyl ether administration might be the result of enhanced testosterone metabolism and subsequent clearance.

No evidence of hepatic peroxisome proliferation was observed in male rats administered methyl t-butyl ether via gavage at 800 mg/kg-day for 14 days (de Peyster et al., 2003). Hepatic nuclear condensation, fat droplets, lysosome appearance in hepatocytes, and smooth endoplasmic reticulum disintegration were observed after subchronic gavage doses of 143 mg/kg-day and higher (Zhou and Ye, 1999). Recent *in vivo* metabolism data indicate that oral exposure to methyl t-butyl ether induces various CYP450 isozymes. Thus, based on the CYP450 data and the lack of reported hepatic histopathology in the chronic gavage study by Belpoggi et al. (1995), the weight of evidence with respect to hepatotoxicity suggests that although methyl t-butyl ether induces various CYP450 isozymes, which may lead to centrilobular hepatocyte hypertrophy, the effect does not progress upon chronic oral exposure. The effects are likely an adaptive mechanism by the liver to metabolizing high doses of methyl t-butyl ether and are likely reversible upon discontinuation of exposure. However, it must be noted that a critical assessment of the progression of the liver effects from short-term or subchronic-to-chronic exposures could not be made, since the non-neoplastic data from Belpoggi et al. (1995) were not available for review, although the authors indicated that no non-neoplastic effects were observed. ECB (2002) concluded that the liver weight increases, hepatic hypertrophy, and changes in smooth endoplasmic reticulum observed after repeated oral exposures to methyl t-butyl ether are typical of other chemicals that are considered to cause adaptive, but reversible, responses by the liver in order to metabolize the chemical.

All investigations on nephrotoxicity associated with methyl t-butyl ether exposure in laboratory rats are consistent with ∀-2μ-globulin nephropathy (IPCS, 1998). ∀-2μ-Globulin nephropathy is considered an effect specific to male rats and, therefore, of questionable relevance to humans.

9.1.2 Weight-of-Evidence Evaluation and Cancer Characterization

Chronic gavage exposure to methyl t-butyl ether was associated with an increase in Leydig cell tumors in male rats and leukemias/lymphomas (combined) in female rats (Belpoggi et al., 1995; 1997; 1998). Although there are no chronic data in humans, there is "*suggestive evidence of carcinogenic potential*" after chronic oral exposure to methyl t-butyl ether in rats.

The genotoxicity data for methyl t-butyl ether have been critically reviewed by ECB (2002), OEHHA (1999), IPCS (1998), ECETOC (1997), and ATSDR (1996). The weight of evidence suggests that methyl t-butyl ether has some genotoxic potential. Methyl t-butyl ether has been tested in mutagenicity, chromosomal aberration, micronucleus, sister chromatid exchange, DNA damage and repair, and DNA strand break assays *in vivo* and/or *in vitro*. Methyl t-butyl ether was not mutagenic in several *Salmonella* reverse mutation assays, although one assay was positive in TA102. Subsequently, a well-conducted study failed to replicate the positive result for methyl t-butyl ether in *Salmonella* TA102 (McGregor et al., 2005). Methyl t-butyl ether was also positive in a mouse lymphoma cell forward mutation assay, possibly due to the metabolism of methyl t-butyl ether to formaldehyde. Methyl t-butyl ether was negative in *in vivo* and *in vitro* chromosomal aberration assays, but equivocal results were observed in a sister chromatid exchange assay *in vitro*. Methyl t-butyl ether was negative in *in vivo* and *in vitro* mouse micronucleus assays and unscheduled DNA synthesis assays, and an *in vivo* DNA repair assay, although methyl t-butyl ether was positive in a DNA strand break assay in rat lymphocytes *in vivo* and in human lymphocytes *in vitro* (Chen et al., 2007). DNA adduct formation was observed in mice given a single gavage dose of methyl t-butyl ether (Du et al., 2005; Yuan et al., 2007).

9.1.3 Mode of Action (Carcinogenic Effects)

The mode of action of the Leydig cell tumors in rats is unclear. There are plausible mechanisms for the chemical induction of Leydig cell tumors, as typified by agonists of estrogen, gonadotropin releasing hormone (GnRH), and dopamine receptors, androgen receptor antagonists, and inhibitors of 5α-reductase, testosterone biosynthesis, and aromatase (Cook et al., 1999). Most of these ultimately involve elevation in serum luteinizing hormone and/or Leydig cell responsiveness to luteinizing hormone. The pathways for regulation of the hypothalamo-pituitary-testis axis of rats and humans are similar, such that compounds that either decrease testosterone or estradiol levels or their recognition will increase luteinizing hormone levels.

Compounds that induce Leydig cell tumors in rats by disruption of the hypothalamo-pituitary-testis axis pose a risk to human health (Cook et al., 1999). However, several lines of evidence suggest that human Leydig cells are quantitatively less sensitive than rats in their proliferative response to luteinizing hormone, and hence in their sensitivity to chemically induced Leydig cell tumors (Cook et al., 1999). This evidence includes the following: (1) the human incidence of Leydig cell tumors is much lower than in rodents even when corrected for detection bias; (2) several comparative differences exist between rat and human Leydig cells that may contribute, at least in part, to the greater susceptibility of the rat to both spontaneous and xenobiotic-induced Leydig cell tumors; (3) endocrine disease states in humans (such as androgen-insensitivity syndrome and familial male precocious puberty) underscore the marked comparative differences that exist between rats and humans in the responsiveness of their Leydig cells to proliferative stimuli; and (4) several human epidemiology studies are available on a number of compounds that induce Leydig cell tumors in rats, such as 1,3-butadiene, cadmium, ethanol, lactose, lead, and nicotine, and that demonstrate no association between human exposure to these compounds and induction of Leydig cell hyperplasia or adenomas (Cook et al., 1999).

Although endocrine-mediated modes of action have been suggested for the induction of testicular tumors by methyl t-butyl ether in rats, OEHHA (1999) and ECB (2002) felt there were insufficient data to support these hypotheses. Based on the available evidence, it seems that the typical mode of action for Leydig cell tumors, which involves elevated luteinizing hormone, is not the case for methyl t-butyl ether (ECB, 2002). Further, testicular cancer is a relatively uncommon cancer in humans. Most human testicular cancers originate either from germ or from Sertoli cells. Tumors of the testes constitute about 1% of all human neoplasm; only 2-3% of all testicular tumors are of Leydig cell origin. Further, methyl t-butyl ether-induced Leydig cell tumors appear in rats only at high doses. ECB (2002) concluded that no definitive conclusion could be drawn about the relevance of the Leydig tumors to humans due to the lack of knowledge of the possible mode of action. Considering all the available data, the relevance to man was not considered very significant by ECB (2002).

In the chronic gavage study by Belpoggi et al. (1995), a reduced incidence of mammary tumors was reported. Such a tumor profile in male and female rats would suggest reduced serum estradiol levels or reduced prolaction secretion, which would cause luteinizing hormone-receptor down-regulation and a subsequent increase in luteinizing hormone (Cook et al., 1999). However, prolactin receptors are either not expressed or are expressed at very low levels in the testes in humans, and thus the induction of Leydig cell tumors in rats by dopamine agonists would appear not to be relevant to humans (Cook et al. 1999).

Methyl t-butyl ether induced mild increases in testosterone hydroxylase enzymes, suggesting that the decrease in serum testosterone observed following repeated oral exposures to methyl t-butyl ether in rats might be the result of enhanced testosterone metabolism and subsequent clearance. Williams et al. (2000) reported decreased serum testosterone and luteinizing hormone after 15- but not 28-day gavage exposures to methyl t-butyl ether, but concluded that these changes in hormone levels did not fit the pattern caused by known Leydig cell tumorigens.

Peroxisome proliferating chemicals have also been known to cause Leydig cell tumors (Klaunig et al., 2003). Peroxisome proliferating chemicals are nongenotoxic carcinogens that mediate their actions through the peroxisome proliferator receptor α. Klaunig et al. (2003) postulated that one mechanism of Leydig cell tumorigenesis begins with peroxisome proliferator receptor α activation in the liver, followed by two possible pathways--one secondary to liver induction and the other direct inhibition of testicular testosterone biosynthesis. Both proposed pathways involved changes in the metabolism and quantity of related hormones and hormone precursors. Klaunig et al. (2003) however, noted that rodents are more responsive than primates in their response to peroxisome proliferators *in vivo*. When de Peyster et al. (2003) administered methyl t-butyl ether at 800 mg/kg-day to rats for two weeks, no effects on hepatic clinical chemistry or peroxisomal proliferation were observed.

The mode of action of the lymphohematopoietic cancers observed in female rats after chronic gavage exposures is unknown. However, the proposed metabolite of methyl t-butyl ether, formaldehyde, has also produced lymphohematopoietic cancers in Sprague-Dawley rats exposed orally (OEHHA, 1998).

9.1.4 Selection of Key Study and Critical Effect

The key study was considered the chronic gavage study by Belpoggi et al. (1995) in which Leydig cell tumors in male rats and hemolymphoreticular leukemias/lymphomas (combined) in female rats were observed at statistically increased incidence compared to controls. Although the study did not meet current U.S. EPA (2007b) Health Effects Testing Guidelines and the study results may have been confounded by early mortality in control and treated rats, it was considered to be adequate for use in a lifetime oral risk assessment for methyl t-butyl ether, since it was conducted by the most appropriate route and duration for a lifetime oral risk assessment for methyl t-butyl ether.

Carcinogenicity of methyl t-butyl ether has been observed after oral and inhalation administration in laboratory animals. Other than the tumors observed in Sprague-Dawley rats from the chronic gavage study by Belpoggi et al. (1995; 1997; 1998), renal tubular tumors and Leydig interstitial cell tumors were observed in male Fischer 344 rats in a 24-month inhalation study (Chun et al., 1992; Bird et al., 1997), and hepatocellular adenomas and/or carcinomas were observed in male and female CD-1 mice in an 18-month inhalation study (Burleigh-Flayer et al., 1992; Bird et al., 1997). The leukemia/lymphomas were not observed consistently in the after inhalation exposure in rats (IPCS, 1998). Increases in Leydig cell tumors occurred at the highest gavage dose (537 mg/kg-day) in Sprague-Dawley rats, but interpretation of the increases in Fischer-344 rats after inhalation exposure was complicated by the very high concurrent and historical control incidences (IPCS, 1998).

The historical control incidence of Leydig cell tumors from studies conducted by Belpoggi were not reported. The historical control incidence of Leydig cell tumors in male rats of various strains has been reported by Cook et al. (1999). In male Sprague-Dawley rats, Leydig cell adenomas were observed in 16/349 (4.8%) of control rats in studies terminated at 24 months (Cook et al., 1999). Other laboratories using Sprague-Dawley rats in 24-month studies have reported historical control incidences of 11/340 (0.8%) and 1/340 (0.1%) for Leydig cell adenomas and carcinomas, respectively (Cook et al., 1999). The route of administration for these studies was not indicated. These historical control incidences are below those observed in control, low- and high-dose males from the Belpoggi et al. (1995) gavage study with methyl t-butyl ether (12, 20, and 34%, respectively).

In male Fischer 344 rats, Leydig cell adenomas were observed in 39,253/51,230 (76.6%) of control rats in studies terminated at 24 months (Cook et al., 1999). The route of administration for these studies was not indicated. This incidence is similar to those observed in control, low-, mid- and high-dose males from the 24-month inhalation study with methyl t-butyl ether (Chun et al., 1992; Bird et al., 1997) (64, 70, 82, and 94%, respectively).

The historical control incidence for lymphomas and leukemias (combined) in the Belpoggi laboratory was reported to be less than 10% in female Sprague-Dawley rats (Belpoggi et al., 1995). This historical control incidence is below the incidence of lymphomas and leukemias (combined) observed in control, low- and high-dose females from the Belpoggi et al. (1995) study with methyl t-butyl ether (3, 14, and 26%, respectively).

In the Belpoggi et al. (1995) study, the animals were observed until natural death, although treatment ended at 104 weeks. Survival at 104 weeks was less than 50%, which is the minimum recommended survival rate according to current U.S. EPA (2007b) Health Effects Testing Guidelines. Survival at the end of treatment was 35% and 28% in low- and high-dose females, respectively, compared to 48% in controls. Survival at the end of treatment was 30% and 42% in low- and high-dose males, respectively, compared to 30% in controls. Further, this study does not meet current U.S. EPA (2007b) Health Effects Testing Guidelines since the dosing occurred on a four-day per week schedule and no results for hematology, clinical chemistry, urinalysis, or organ weights were reported.

Recognizing the deficiencies in the Belpoggi et al. (1995) study, using a chronic oral study to estimate lifetime cancer risk from oral exposure to methyl t-butyl ether was considered more appropriate than using a chronic inhalation study and conducting an inhalation-route-to-oral-route extrapolation to estimate a lifetime cancer risk from oral exposure to methyl t-butyl ether. Further, the historical control incidences for Leydig cell adenomas as reported by Cook et al. (1999) were well below those observed in control, low- and high-dose males from the Belpoggi et al. (1995) gavage study. Likewise, the historical control incidence for lymphomas and leukemias (combined) in the Belpoggi laboratory were below those observed in control, low- and high-dose females from the Belpoggi et al. (1995) gavage study with methyl t-butyl ether. However, as reported by IPCS (1998), the diagnostic criteria for the distinction between Leydig cell tumors and hyperplasia were not indicated by Belpoggi et al. (1995; 1997; 1998), and the latter were not reported at all, which was considered unusual by IPCS (1998) for old Sprague-Dawley rats showing Leydig cell tumors.

9.1.5 Identification of Susceptible Populations

There are no data by which to identify any subpopulations (e.g., the elderly, pregnant women, children, or people with allergies or asthma) that might be at special risk to methyl t-butyl ether exposure (IPCS, 1998).

9.2 Dose-Response Assessment

For the dose-response assessment, the statistical 95% lower confidence limit of the 10% effect level, also known as the $BMDL_{10}$, was estimated using default settings in the Benchmark Dose Program (Version 1.4.1c, U.S. EPA, 2007a). In the Belpoggi et al. (1995; 1997; 1998) study, Sprague-Dawley rats were administered methyl t-butyl ether via gavage at 0, 250, or 1,000 mg/kg-day for four days a week for 24 months. These doses were approximately equivalent to daily doses of 0, 143, or 571 mg/kg-day. In male rats, the incidence of Leydig cell tumors was statistically increased at the high dose compared to the controls, and there was a statistically significant dose-related trend at the mid and high dose. In female rats, the combined incidence of lymphomas and leukemias was statistically increased at the high dose compared to the controls, and there was a statistically significant dose-related trend at the mid- and high-dose.

9.2.1 Dose-response assessment based on tumor data in male rats

The administered doses in male rats were converted to human equivalent doses of 0, 42.5, or 170 mg/kg-day, based on the following equation.

$$\text{Human Equivalent Dose} = \text{dose (mg/kg-day)} \times (\text{kg wt. rat}/70 \text{ kg wt. human})^{0.25}$$

Mean terminal body weight for low-dose male Sprague-Dawley rats from Belpoggi et al. (1995; 1997; 1998) = 0.550 kg (data not provided; estimated from graph at 104 weeks, time at which dosing ended, although animals were observed until natural death). Mean terminal body weight for high-dose male Sprague-Dawley rats from Belpoggi et al. (1995; 1997; 1998) = 0.550 kg (data not provided; estimated from graph at 104 weeks, time at which dosing ended, although animals were observed until natural death). In male rats, the incidence of Leydig cell tumors was statistically increased at the high dose compared to the controls (Table 3).

Table 3. Leydig cell tumors in male rats after chronic gavage exposure to methyl t-butyl ether (Belpoggi et al., 1995; 1997; 1998)

methyl t-butyl ether dose (mg/kg-day; human equivalent dose)	Incidence of Leydig cell tumors[a]	Incidence of Leydig cell tumors[b]
0	3/26 (12%)	3/37.8 (8%)
42.5	5/25 (20%)	5/37.6 (13%)
170	11/32 (34%)*	11/40.4 (27%)

[a] Number of rats affected/number surviving at appearance of first tumor (96 weeks) based on Belpoggi et al. (1995, 1998)
[b] Number of rats affected/effective number at risk after Poly-3 adjustment for survival based on Kippling et al. (2007)
* $p<0.05$

Since there was a statistically significant dose-related trend at the mid- and high-dose, a benchmark dose level at the lower 95% confidence interval (BMDL) for methyl t-butyl ether will be determined based on the incidence of Leydig cell tumors in male rats. The $BMDL_{10}$ was defined as the lower 95% confidence interval of the dose at which one could expect a 10% increased incidence in a given population (Table 4).

Table 4. Results of benchmark dose modeling of Leydig cell tumors from male rats (all alive at 96 weeks, first observed tumor)

Model	P value	AIC	Chi square residuals	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.8224	88.8501	-0.107, 0.187, -0.063	60	32
Logistic	0.6578	88.9955	-0.278, 0.340, -0.060	83	56
Multistage[1]	NA	90.8002	0, 0, 0	45	12
Probit	0.6760	88.9739	-0.256, 0.324, -0.063	80	53
Quantal Linear/Weibull[2]	0.8224	88.8501	-0.107, 0.187, -0.063	60	32

P value = Global measurement of goodness-of-fit (P > 0.1)
AIC (Akaike's Information Criterion) = Model comparison (lowest value preferred)
Chi square = Local measurement of goodness-of-fit (< |2.0|).
[1] Multistage Cancer BMDU = 279 mg/kg-day (restrict beta) and Cancer Slope Factor = 0.0032
[2] BMD program defaulted Quantal Linear model to Weibull model

The Gamma, Quantal Linear and Weibull models provided the best fit, based on the highest p value, lowest AIC value, local Chi^2 values of less than the absolute value of two for all data points, and a good fit of both the BMD and BMDL dose-response plots (see Appendix). The calculated BMD (60 mg/kg-day) and BMDL (32 mg/kg-day) were in close approximation of each other.

9.2.2 Dose-response assessment based on tumor data in female rats

Alternately, the $BMDL_{10}$ for methyl t-butyl ether can be calculated based on the combined incidence of lymphomas and leukemias in female rats from the Belpoggi et al. (1995; 1997; 1998) study. The doses were equivalent to human oral doses of 0, 38.7, or 152 mg/kg-day, based on the following equation.

$$\text{Human Equivalent Dose} = \text{dose (mg/kg-day)} \times (\text{kg wt. rat}/70 \text{ kg wt. human})^{0.25}$$

Mean terminal body weight for low-dose female Sprague-Dawley rats from Belpoggi et al. (1995; 1997; 1998) = 0.375 kg (data not provided; estimated from graph at 104 weeks, time at which dosing ended, although animals were observed until natural death). Mean terminal body weight for high-dose female Sprague-Dawley rats from Belpoggi et al. (1995; 1997; 1998) = 0.355 kg (data not provided; estimated from graph at 104 weeks, time at which dosing ended, although animals were observed until natural death). In female rats, the combined incidence of lymphomas and leukemias was statistically increased at the high dose compared to the controls (Table 5).

Table 5. Combined incidence of hemolymphoreticular lymphomas and leukemias in female rats after chronic gavage exposure to methyl t-butyl ether (Belpoggi et al., 1995; 1997; 1998)

methyl t-butyl ether dose (mg/kg-day human equivalent dose)	Incidence of hemolymphoreticular lymphomas and leukemias [a]
0	2/58 (3%)
38.7	7/51 (14%)
152	12/47 (26%)*

[a] Number of rats affected/number surviving at appearance of first tumor (56 weeks)
* $p<0.05$

Since there was a statistically significant dose-related trend at the mid- and high-dose, a benchmark dose level at the lower 95% confidence interval ($BMDL_{10}$) for methyl t-butyl ether will be determined based on the combined incidence of lymphomas and leukemias in female rats (Table 6).

Table 6. Results of benchmark dose modeling of hemolymphoreticular lymphomas and leukemias from female rats (all alive at 56 weeks, first observed tumor)

Model	P value	AIC	Chi square residuals	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.4246	116.208	-0.284, 0.675, -0.318	57	36
Logistic	0.1604	117.587	-0.894, 1.066, -0.186	91	70
Multistage[1]	NA	117.596	0, 0, -0	36	16
Probit	0.1779	117.413	-0.831, 1.039, -0.213	87	65
Quantal Linear/Weibull[2]	0.4246	116.208	-0.284, 0.675, -0.318	57	36

P value = Global measurement of goodness-of-fit (P > 0.1)
AIC (Akaike's Information Criterion) = Model comparison (lowest value preferred)
Chi square = Local measurement of goodness-of-fit (< |2.0|).
[1] Multistage Cancer BMDU = 279 mg/kg-day (restrict beta) and Cancer Slope Factor = 0.0032
[2] BMD program defaulted Quantal Linear model to Weibull model

The Gamma, Quantal Linear and Weibull models provided the best fit, based on the highest p value, lowest AIC value, local Chi^2 values of less than the absolute value of two for all data points, and a good fit of both the BMD and BMDL dose-response plots (see Appendix). The calculated BMD (57 mg/kg-day) and BMDL (36 mg/kg-day) were in close approximation of each other.

9.2.3 Oral Slope Factor Calculation

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There were insufficient data to support a mode of action for the Leydig cell or lymphatic tumors. In the absence of mode of action information, the U.S. EPA (2003) generally takes a conservative, or public health-protective, default position regarding the interpretation of toxicological data. This conservative approach assumes that the animal tumor findings are relevant to humans and that cancer risks are assumed to conform with low dose linearity (U.S. EPA, 2003). Elucidation of a mode of action for a particular cancer response in animals or humans is a data-rich determination. Significant information should be developed to ensure that a mode of action underlies the process leading to cancer at a given site (U.S. EPA, 2003). Based on this approach, both the lymphatic and Leydig cell tumors in rats were assumed to be relevant to humans and the associated cancer risks were assumed to conform to low dose linearity. Thus, a 10^{-5} risk level will be calculated for methyl t-butyl ether based on the $BMDL_{10}$ of 32 mg/kg-day (the lower of the two $BMDL_{10}$ values, recognizing that they were both essentially the same). The slope of the dose-response line, known as the slope factor, is an upper-bound estimate of risk per increment of dose that can be used to estimate risk probabilities for different exposure levels (U.S. EPA, 2003c). The slope factor is equal to $0.01/LED_{01}$ if the LED_{01} is used as the point of departure (U.S. EPA, 2003c). Since the 10% benchmark dose level was used in this risk assessment, then the oral slope factor was determined according to the following equation.

$$\text{Oral Slope Factor} = \frac{0.1}{BMDL_{10}}$$

$$\text{Oral Slope Factor} = \frac{0.1}{32 \text{ mg/kg-day}}$$

Oral Slope Factor = 0.003125 $(mg/kg\text{-}day)^{-1}$

The Cancer Slope Factor of 0.003125 $(mg/kg\text{-}day)^{-1}$ as estimated by NSF International is essentially the same as the Cancer Slope Factor of 0.0032 $(mg/kg\text{-}day)^{-1}$ estimated by the Multistage model of the Benchmark Dose Software Version 1.4.1c (U.S. EPA, 2007a). A 10^{-5} (or 1 in 100,000) cancer risk level can determined from the $BMDL_{10}$ according to the following linear extrapolation:

$$\frac{0.1}{BMDL_{10}} = \frac{0.00001}{10^{-5}\text{ risk level}}$$

$$\frac{0.1}{32\text{ mg/kg-day}} = \frac{0.00001}{10^{-5}\text{ risk level}}$$

10^{-5} risk level = 0.003125 mg/kg-day

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9.3.3.1 Drinking Water Unit Risk Calculation

The unit risk, defined as the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 µg/L (U.S. EPA, 2003a), may be calculated from the slope factor. Risk-specific doses are derived from the slope factor or unit risk to estimate the dose associated with a specific risk level, for example, a one-in-a-million increased lifetime risk (U.S. E.P.A., 2003c). The unit risk is calculated from the slope factor using the default 70 kg body weight and 2 L/day drinking water consumption of an adult:

$$\text{Unit Risk} = \frac{0.003125\text{ kg-day}}{\text{mg}} \times \frac{1}{70\text{ kg}} \times \frac{2\text{ L}}{\text{day}} \times \frac{1\text{ mg}}{1{,}000\text{ µg}} = 9.0 \times 10^{-8}\ (\mu g/L)^{-1}$$

$$= 0.009 \times 10^{-5}\ (\mu g/L)^{-1}$$

or

$$= 0.09 \times 10^{-6}\ (\mu g/L)^{-1}$$

Therefore, drinking water containing 1 µg/L of methyl t-butyl ether consumed for a lifetime is estimated to result in development of 0.009 excess tumors per 100,000 people, and drinking water containing 1 µg/L of methyl t-butyl ether consumed for a lifetime is estimated to result in development of 0.09 excess tumors per 1,000,000 people. Alternately, drinking water containing 90 µg/L of methyl t-butyl ether consumed for a lifetime is estimated to result in development of 1 excess tumor per 100,000 people, or drinking water containing 9 µg/L of methyl t-butyl ether consumed for a lifetime is estimated to result in development of 1 excess tumor per 1,000,000 people.

9.3 Exposure Assessment

The presence of methyl t-butyl ether in ambient air as a result of the manufacture and distribution of oxygenated fuel, vehicle refueling processes, and evaporative and tailpipe emissions from motor vehicles, is likely to be the principal source of human exposure (OEHHA, 1999). Methyl t-butyl ether is infrequently detected in public drinking water systems from groundwater (IPCS, 1998). There are inadequate data to characterize the concentration of methyl t-butyl ether in public drinking water systems from surface water. Methyl t-butyl ether has been found at high levels (i.e. ≥ 1,000 µg/L) in a few private wells used for drinking water (IPCS, 1998). Exposure of the public to methyl t-butyl ether can be principally by inhalation of fumes while refueling motor vehicles and drinking contaminated water (McGregor, 2006). Maximum internal doses resulting from such exposures are unlikely to exceed 0.05 mg/kg-day and will normally be very much lower.

9.4 TAC Derivation

The Total Allowable Concentration (TAC), is used to evaluate the results of extraction testing normalized to static at-the-tap conditions and is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), applied when calculating a TAC for non-carcinogens, is used to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered. Since the TAC value for methyl t-butyl ether is based on a carcinogenic endpoint, a RSC will not be applied. The TAC for methyl t-butyl ether will be set to the 10^{-5} cancer risk level for methyl t-butyl ether.

$$\text{TAC} = \frac{10^{-5}\text{ risk level} \times 70\text{ kg}}{2\text{ L/day}}$$

$$= \frac{(0.003\text{ mg/kg-day})(70\text{ kg})}{2\text{ L/day}}$$

$$= 0.105\text{ mg/L } (\textbf{100 ppb rounded})$$

9.5 STEL Derivation

NSF/ANSI 60 (2005) and 61 (2007) allow for the derivation and use of a STEL for materials that are initially present in potable water at relatively high concentrations, but rapidly decline in concentration because they are volatile or because they chemically or biologically degrade. The STEL is generally calculated from a repeated dose study in laboratory animals of 14 to 90 days in duration, adjusted for the default 10 kg body weight and 1 L/day drinking water consumption of a child. A product can initially contribute up to the STEL if the at-the-tap concentration decreases to a level at or below the TAC or SPAC within 90 days. Since methyl t-butyl ether is being evaluated as a genotoxic carcinogen, exposure to drinking water levels higher than the TAC, set at the 10^{-5} risk level, cannot be justified and it is not appropriate to derive a STEL for this chemical.

10.0 RISK MANAGEMENT

10.1 SPAC Derivation

The SPAC is set at the 10^{-6} cancer risk level of 0.01 mg/L (or 100 ppb) for methyl t-butyl ether. This is based on the default 10 sources of the chemical in the water distribution system in the absence of data on the actual number of sources.

11.0 RISK COMPARISONS AND CONCLUSIONS

The scientific literature for methyl t-butyl ether in humans and laboratory animals has been reviewed extensively by several national and international regulatory agencies. Table 7 provides a summary of the most recent and major reviews along with the major conclusions from each assessment regarding the non-cancer and cancer human health risks from exposure to methyl t-butyl ether.

Although the conclusions from the various agencies regarding non-cancer effects are largely in agreement with each other, the conclusions regarding the carcinogenic potential in rats after oral exposure are divergent. The American Conference of Governmental Industrial Hygienists (ACGIH, 2005) has classified methyl t-butyl ether as a Class A3: Animal Carcinogen. The International Agency for Research on Cancer (IARC) has reported that there is limited evidence in humans and in experimental animals for the carcinogenicity of methyl t-butyl ether. Thus, it was concluded by IARC (1999) that methyl t-butyl ether is not classifiable as to its carcinogenicity to humans (Group 3).

Based on a critical review of the genotoxicity data for methyl t-butyl ether, ECB (2002) concluded that methyl t-butyl ether cannot be considered a mutagen. OEHHA (1999) concluded that that the data are weak and there is no clear evidence that methyl t-butyl ether or its metabolites are involved in the carcinogenic response in laboratory animals. IPCS (1998) concluded that the weight of evidence suggests that methyl t-butyl ether is not genotoxic. ECETOC (2003 and 1997) concluded that genotoxicity of methyl t-butyl ether is unlikely to play a role in neoplastic findings reported in chronic studies with methyl t-butyl ether. Further, the inhaled concentrations causing neoplastic effects are equal to or greater than those inducing non-neoplastic effects in female mouse liver and male rat kidney. Thus, protection against non-neoplastic effects should also protect from any theoretical carcinogenic effect (ECETOC (2003).

1 **Table 7. Summary of international and national regulatory risk assessments or literature reviews for methyl t-butyl ether**

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Organization/ Reference	Month/ Year	Conclusions from Non-Cancer Assessment of Oral Exposure	Conclusions from Cancer Assessment of Oral Exposure	Regulatory Risk Level Derived?
Caldwell et al. (2007)	12/2007	Not assessed	Hemolymphoreticular tumors of Belpoggi et al., (1995) were considered to be exposure-related and relevant to humans.	No
Cruzan et al. (2007)	2007	Not assessed	There were weak tumorigenic response in the testes and kidneys of male rats and liver in female mice. The weight of the evidence does not support a genotoxic mode of action. Non-genotoxic mode of actions have been demonstrated or suggested that correspond to the weak tumorigenic responses. These mode of actions either do not occur in humans or humans are much less susceptible to these. It is, therefore, unlikely that humans would be exposed to suffcient levels of methyl t-butyl ether to cause these tumorigenic responses.	No
McGregor, 2006	2006	Not assessed	Evidence for carcinogenicity in rodents of methyl t-butyl ether or it's metabolite t-butanol is unconvincing, with the strongest being for a low-level incidence of renal tubule-cell adenomas by a mechanism that is specific to male rats and has no human relevance.	No
American Conference of Governmental Industrial Hygienists (ACGIH, 2005)	2005	Reproductive and kidney effects were reported but not specified.	Classified methyl t-butyl ether as a Class A3: Animal Carcinogen (classification not route-specific)	No
National Institute of Public Health & Environmental Protection, The Netherlands (Baars, 2004; RIVM, 2004)	11/2004	RIVM (2004) adopted the conclusions of ECB (2002)		0.3 mg/kg-day tolerable daily intake (non-cancer)
European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2003 and 1997)	12/2003 and 6/1997	Non-cancer conclusions of oral exposure were not indicated.	Methyl t-butyl ether was not considered carcinogenic. Rat Leydig cell tumors after chronic gavage were not considered predictive of hazard to humans. Further, the importance of the combined lymphoma/leukemia incidence from this study was considered to be unclear due to deficiencies in the study report.	No

Organization/ Reference	Month/ Year	Conclusions from Non-Cancer Assessment of Oral Exposure	Conclusions from Cancer Assessment of Oral Exposure	Regulatory Risk Level Derived?
European Chemicals Bureau (ECB, 2002)	9/2002	Based on the effects in the rat "liver" from the 90-day gavage study by Robinson et al. (1990), the subchronic NOAEL was considered 300 mg/kg-day. Note that it is presumed by the authors of this risk assessment that ECB (2002) was referring to the effects in the rat kidney, rather than liver. The chronic oral LOAEL was considered 250 mg/kg (or 143 mg/kg-day; see cancer assessment)	Due to lack of direct evidence of genotoxicity, the chronic oral LOAEL was considered 250 mg/kg (143 mg/kg-day) based on the Leydig cell tumors in male rats and lymphatic tumors in female rats, recognizing limitations and uncertainties due to the inadequacies of the chronic oral study by Belpoggi et al. (1995). The LOAEL of 250 mg/kg with a total daily uptake of 0.0021 mg/kg determined the margin of safety to be 125,000. Methyl t-butyl ether was considered borderline between a Carcinogen Category of 3 or non-classification.	No
International Agency for Research on Cancer (IARC, 1999)	9/1999	Not assessed.	Methyl t-butyl ether was tested for carcinogenicity in a non-standard protocol in rats by gavage. The incidences of Leydig-cell tumors of the testis in males and of lymphomas and leukemias combined in females were increased. There is *inadequate evidence* in humans for the carcinogenicity of methyl *t*-butyl ether. There is *limited evidence* in experimental animals for the carcinogenicity of methyl *t*-butyl ether (note that conclusions were not route-specific). Thus, methyl *t*-butyl ether is *not classifiable as to its carcinogenicity to humans (Group 3)*.	No
California EPA Office of Environmental Health Hazard Assessment (OEHHA, 1999)	3/1999	Based on the effects in the rat kidney from the 90-day gavage study by Robinson et al. (1990), the oral NOAEL was considered 100 mg/kg-day. This study was used to determine a public health goal for non-cancer effects of 47 ppb in drinking water.	Methyl t-butyl ether is an animal carcinogen and possible human carcinogen (note that conclusion was not route-specific). The Belpoggi et al. (1995) study was determined to be adequate for risk assessment purposes. This study, along with two chronic inhalation studies, was used to determine a public health goal for cancer effects of 13 ppb in drinking water (based on 10^{-6} risk level and 3 L/day water intake).	47 ppb in drinking water (non-cancer); 13 ppb in drinking water (cancer)

Organization/ Reference	Month/ Year	Conclusions from Non-Cancer Assessment of Oral Exposure	Conclusions from Cancer Assessment of Oral Exposure	Regulatory Risk Level Derived?
WHO International Programme on Chemical Safety (IPCS, 1998)	1998	Based on the increased male absolute and relative kidney weight, chronic nephropathy, and increase in hyaline droplets in proximal tubular cells effects in the rat liver from the 90-day gavage study by Robinson et al. (1990), the subchronic oral NOAEL was considered 300 mg/kg. The chronic oral LOEL was considered 1,000 since no adverse non-neoplastic effects were reported by Belpoggi et al. (1995). A chronic NOAEL or LOAEL for non-cancer effects was not identified.	Leydig cell tumors in rats have been induced by non-genotoxic carcinogens that disturb the hormonal balance of testosterone, luteinizing hormone, and luteinizing hormone-releasing factor in rats. Owing to differences between rats and humans in the regulation of gonadotropins, it is questionable that a similar effect will occur in humans. Although such a mechanism may be relevant, this is not substantiated by experimental evidence, since these hormones were not determined in any of the studies with methyl t-butyl ether. In female rats, lymphomas and leukemias (combined) were increased. This observation was not supported by preneoplastic effects on the lymphoid system in other studies. Moreover, the study description made it difficult to evaluate adequately the results. However, since the effect appeared rather pronounced, it is not justified to neglect it, based on presumed experimental deficiencies. For a proper evaluation, additional information is required. Thus, methyl t-butyl ether should be considered a rodent carcinogen at high doses, which also induces other adverse effects. The available data are inconclusive and prohibit its use for human carcinogenic risk assessment until outstanding complications in its interpretation have been addressed.	No
U.S. Environmental Protection Agency (US EPA, 1997)	12/1997	The recommended level in drinking water of 20-40 ppb based on averting taste and odor provides a sufficient margin of exposure for cancer and non-cancer effects observed in laboratory animals. A level based on cancer risk level or a non-cancer RfD was not developed due to data limitations.		20-40 ppb in drinking water
Agency for Toxic Substances and Disease Registry (ATSDR, 1996)	8/1996	An acute minimum risk level was based on a 40 mg/kg NOAEL for lack of drowsiness in rats after a single gavage dose in a study by Bioresearch Laboratories (1990). An intermediate minimum risk level was based on a 100 mg/kg LOAEL for decreased blood urea nitrogen in rats from the Robinson et al. (1990) subchronic gavage study. (1990). Chronic minimal risk level (> 365 days) was not determined, since chronic oral exposure in female rats was associated with increased mortality and lymphatic tumors.	Chronic minimal risk level (> 365 days) was not determined for methyl t-butyl ether, since chronic oral exposure in female rats was associated with increased mortality and lymphatic tumors.	Minimum risk levels of 0.4 mg/kg-day for acute exposures (<14 days); 0.3 mg/kg-day for intermediate duration exposures (15-364 days)

Organization/ Reference	Month/ Year	Conclusions from Non-Cancer Assessment of Oral Exposure	Conclusions from Cancer Assessment of Oral Exposure	Regulatory Risk Level Derived?
Health Canada (1992; 1996)	1992; 1996	The predicted concentrations of methyl t-butyl ether in the Canadian environment do not constitute a danger to human life or health. Methyl t-butyl ether is not considered to be "toxic" as defined under Section 11 of the Canadian Environmental Protection Act (Health Canada, 1992).	Carcinogenicity Class VI. Tolerable Daily Intake for cancer effects not determined (Health Canada, 1996).	0.01 mg/kg-day Oral Tolerable Daily Intake for non-cancer effects.

Since the Belpoggi et al. (1995) chronic gavage study in rats was not designed to meet a standardized protocol, the study was considered by some regulatory agencies to have deficiencies that would impact a human health risk assessment for methyl t-butyl ether. In summary, some agencies or organizations considered the Belpoggi et al. (1995) study to be inadequate for a human health risk assessment for oral exposure while other agencies considered the study to be adequate, although flawed.

Among the agencies or organizations that considered the Belpoggi et al. (1995) study or the overall evidence to be inadequate to assess the carcinogenic potential in humans from oral exposure to methyl t-butyl ether were the European Chemicals Bureau (ECB, 2002), the International Agency for Research on Cancer (IARC, 1999), the International Programme on Chemical Safety (IPCS, 1998), and the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2003 and 1997). IPCS (1998) critically reviewed the Belpoggi et al. (1995) study and noted several confounding factors, such as:

(1) there is limited description of the results, particularly the histopathological findings;
(2) diagnostic criteria are not given for the distinction between Leydig cell tumors and hyperplasia (the latter were not reported at all, which is unusual for old Sprague-Dawley rats showing Leydig cell tumors);
(3) diagnostic criteria are not given for the distinction between dysplastic hyperplasia and lymphoma;
(4) lymphomas and leukemias are pooled; specific tumor type and incidences were not reported;
(5) historical control data might aid the evaluation of lymphomas and leukemias, particularly if they are available for these rats within different age ranges; and
(6) chronic progressive nephropathy was not observed in these Sprague-Dawley rats, although these lesions might be expected, on the basis of data from a number of other studies with this strain of rat.

IPCS (1998) concluded that owing to differences between rats and humans in the regulation of gonadotropins, it is questionable that the Leydig cell tumors observed in rats would be seen in humans. IPCS (1998) further indicated that these tumors have been reported to be induced by non-genotoxic carcinogens that disturb the hormonal balance of testosterone, luteinizing hormone, and luteinizing hormone releasing factor in rats, and that although such a mechanism may be relevant in humans, it was not substantiated by experimental evidence, since these hormones were not determined in any of the studies with methyl t-butyl ether (IPCS, 1998). However, since the IPSC review, recent *in vivo* and *in vitro* studies by de Peyster et al. (2003) have demonstrated that single and repeated gavage exposures to methyl t-butyl ether can reduce circulating testosterone levels. Further, studies by Williams et al. (2000) indicated that methyl t-butyl ether can induce mild increases in testosterone hydroxylase enzymes, suggesting that the decrease in serum testosterone observed following repeated oral exposures to methyl t-butyl ether in rats may be the result of enhanced testosterone metabolism and subsequent clearance. However, Williams et al. (2000) noted that the changes in testosterone and luteinizing hormone levels after short-term gavage exposures to methyl t-butyl ether in rats did not fit the pattern caused by known Leydig cell tumorigens.

US EPA (1997) recommended a drinking water level for methyl t-butyl ether of 20-40 ppb based on averting taste and odor, and considered this level to provide a sufficient margin of exposure for cancer and non-cancer effects observed in laboratory animals. Under the U.S. EPA (2008) IRIS Program, the draft re-assessment of methyl t-butyl ether is currently undergoing the "Agency Review" step with an estimated completion date of February 17, 2008. The next stages are "Interagency Review" and "External Peer Review".

The National Institute of Public Health and Environmental Protection of the Netherlands (Baars, 2004; RIVM, 2004), Agency for Toxic Substances and Disease Registry (ATSDR, 1996) and Health Canada (1996; 1991) have not derived chronic regulatory values for methyl t-butyl ether based on cancer effects, although values for less than chronic exposure and/or non-cancer effects have been derived (Table 7). RIVM (2004; Baars, 2004) considered the subchronic gavage study by Robinson et al. (1990) as the key study and applied 1,000x uncertainty factor (10 each for inter- and intraspecies differences, and 10 total for limited duration of the study and database deficiences) to the NOAEL of 300 mg/kg-day to determine a tolerable daily intake of 0.3 mg/kg-day. Note that NSF considered the reduced lung weights treatment-related at all exposure doses in females and thus, could not identify a NOAEL for female rats in this study.

Among the organizations or authors that considered the Belpoggi et al. (1995) study to be adequate, although flawed, to assess the carcinogenic potential in humans from oral exposure to methyl t-butyl ether were Caldwell et al. (2007) and the Office of Environmental Health Hazard Assessment of California EPA (OEHHA, 1999). OEHHA (1999) reported that the National Academy of Sciences (NRC, 1996) reviewed the chronic gavage study by Belpoggi et al. (1995) and noted the following as study deficiencies:

(1) the dosage schedule of Monday, Tuesday, Thursday, and Friday, rather than five consecutive days;
(2) use of doses in apparent excess of the Maximum Tolerated Dose (MTD), based on a dose-related decrease in survival among treated females;
(3) the combining of leukemia and lymphoma incidence;
(4) the incomplete description of tumor pathology and diagnostic criteria; and
(5) the lack of mortality adjusted analysis to account for differences in survival times.

OEHHA (1999) considered these criticisms and concluded that although these experiments, like the others available for methyl t-butyl ether, do have certain limitations or difficulties of interpretation, they contribute considerably to the overall evidence available for methyl t-butyl ether risk assessment. Further, OEHHA (1999) concluded that the study was valid, not critically flawed, and consistent with other reported results. Caldwell et al. (2007) considered the background incidence of hemolymphoreticular tumors in female rats from Belpoggi et al. (1995) to be consistent with other studies. Further, the hemolymphoreticular tumors were considered to be exposure-related, relevant to humans, and unlikely to be due to infections. The review authors also supported the combination of lymphoblastic leukemias and lymphomas reported by Belpoggi et al. (1995) for the purposes of risk assessment.

Goodman et al. (2007) and NTP (Kissling et al., 2007) provided recent insight into the lack of mortality-adjusted analysis to account for differences in survival times noted as a deficiency by NRC (1996). Goodman et al. (2007) do not consider the increase in Leydig cell tumors in treated male rats to be statistically significant when these data are re-evaluated with a statistical adjustment (Poly-3 test) for the increased survival in the high-dose males, which provides an increased opportunity for the occurrence of tumors. However, NTP (Kissling et al., 2007) rebutted that the Poly-3 analysis was developed by the NTP (Bailer and Portier, 1988; Portier and Bailer, 1989) based on survival rates from 104-week (two-year) studies and was not designed to encompass longer-term studies like Belpoggi et al. (1995) in which rats were observed until natural death of up to 174 weeks. Kissling et al. (2007) demonstrated that the increase in Leydig tumors in male rats exposed to methyl t-butyl ether was statistically significant according to the Poly-3 test based on survival at 104 weeks, for which the test was designed. When the Leydig cell tumor data were evaluated in the Poly-3 test based on the exact death times obtained through communications with Dr. Belpoggi, Kissling et al. (2007) reported the incidence of Leydig cell tumors to be 3/37.8 (8%), 5/37.6 (13%), and 11/40.4 (27%) in control, low-, and high-dose rats, respectively, based on the effective number of rats at risk after adjustment for survival at 104 weeks. These data compare to the incidences of 3/26 (12%), 5/25 (20%), and 11/32 (34%), respectively, based on the total number of rats alive at the appearance of the first Leydig tumor (96 weeks) reported by Belopoggi et al. (1995). A $BMDL_{10}$ of 67 mg/kg-day (data not shown) was estimated by NSF International based on the adjusted survival reported by Kissling et al. (2007). Note that the Benchmark Dose program (V.1.4.1c., 2007a) rounds the number of rats in each dose group to 38, 38 and 40, respectively. This $BMDL_{10}$ of 67 mg/kg-day compares to the $BMDL_{10}$ of 32 mg/kg-day based on the survival at 96 weeks, time of first Leydig tumor. The $BMDL_{10}$ for Leydig tumors based on Poly-3-adjusted survival is provided as a comparison only, as no regulatory guidance is available regarding this Poly-3-based approach to estimate the $BMDL_{10}$. Further, the performance of the Poly-3 test depends on how closely it represents the correct specification of the time-at-risk weight in the data (Moon et al., 2003). A similar mortality-adjusted analysis for the lymphatic tumors in female rats was not identified in the published literature.

As noted above, a key difference in the methodology of the Belpoggi et al. (1995) study compared to NTP chronic studies is that the treatment duration and sacrifice time are the same (104 weeks) for NTP rat studies. Whereas, Belpoggi et al. (1995) observed the rats until natural death in order to assess late-appearing tumors, although the treatment duration was 104 weeks. NSF used the total number of rats alive at the appearance of the first Leydig tumor (96 weeks) reported by Belopoggi et al. (1995) in order to estimate the $BMDL_{10}$. NSF did not feel it was appropriate to include all sixty animals per dose in the $BMDL_{10}$ estimation, since the late-appearing Leydig cell tumors may have been spontaneous, particularly considering the high spontaneous incidence of this tumor and the fact that Portier et al. (1986) found spontaneously-occurring Leydig cell tumors in control rats, albeit the F344 strain, to be clearly non-lethal. Note that a $BMDL_{10}$ based on Poly-3 adjustment for survival at 104 weeks for the lymphatic tumors in female rats could not be estimated by NSF International, because survival data at 104 weeks was not reported by Belpoggi et al. (1995).

Two inhalation studies in rats (Burleigh-Flayer et al., 1992; Chun et al., 1992; Bird et al., 1997) and the gavage study (Belpoggi et al. 1995; 1997; 1998) in rats were used by OEHHA (1999) to

develop a public health goal (PHG) of 13 ppb for methyl t-butyl ether in drinking water. The value was derived from the geometric mean of the cancer slope factors of the combined male rat kidney adenomas and carcinomas after inhalation exposure, the male rat Leydig cell tumors after gavage and inhalation exposure, and the leukemia and lymphomas in female rats after gavage exposure. The value was set at the 10^{-6} cancer risk level and assumed three liters of water consumption per day. OEHHA (1999) indicated that while some reviews have given less weight to the Belpoggi et al. (1995; 1997; 1998) studies, OEHHA (1999) found that they contributed to the overall weight of evidence.

OEHHA (1999) did not consider the renal tubule tumors observed in male rats after chronic inhalation of methyl t-butyl ether to be associated with α-2μ-globulin nephropathy, and cited investigations by NSTC (1997) and U.S. EPA (1997). These reviews reported that the possibility of male rat-specific α-2μ-globulin nephropathy playing a significant role in the pathogenesis of methyl t-butyl ether rat kidney tumors is unlikely. According to OEHHA (1999), these reviews conclude that the data indicate only mild accumulation of α-2μ-globulin and mild or partial expression of α-2μ-globulin associated nephropathy in male rats, while clearly exacerbating the expression of non- α-2μ-globulin rat nephropathy in both males and females. Further, a dose-dependent increase in mortality from chronic progressive nephropathy was observed in male rats at all dose levels, and in females at the mid- and high-dose levels in the rat inhalation bioassay by Bird et al. (1997). However, the IPCS (1998) considered all investigations on nephrotoxicity associated with methyl t-butyl ether exposure in laboratory rats to be consistent with α-2μ-globulin nephropathy and of questionable relevance to human health.

Due to the limited oral data for methyl t-butyl ether, Dourson and Felter (1997) reviewed the toxicokinetic data for methyl t-butyl ether and suggested that there are sufficient data to conduct an inhalation-to-oral route extrapolation for methyl t-butyl ether. Based on the two-year inhalation toxicity study by Chun et al. (1992), in which rats were exposed to 0, 400, 3,000, or 8,000 ppm methyl t-butyl ether for six hours per day and five days per week, human equivalent oral doses of 0, 130, 940, or 2,700 mg/kg-day were estimated. These doses were estimated using a physiologically-based pharmacokinetic model that compared the differences between the absorption, distribution, metabolism, and elimination of methyl t-butyl ether after inhalation compared to oral exposure. After a review of the kinetic and metabolism data, Dourson and Felter (1997) concluded that the ratio of inhalation-to-oral absorption was between 0.4 to 1, and thus chose 0.5 for the absorption component of the physiologically-based pharmacokinetic model. It was also concluded that the ratios between inhalation-to- oral distribution, metabolism, and elimination of methyl t-butyl ether in rats were each one, since these parameters were considered equivalent between the inhalation and oral routes. The human equivalent oral doses that were estimated based on the the Chun et al. (1992) chronic inhalation study were proposed for use in oral non-cancer and cancer risk assessments for methyl t-butyl ether. In this study, renal tubular tumors and Leydig interstitial cell tumors were observed at an increased incidence in male rats compared to controls.

Based on the physiologically based pharmacokinetic model proposed by Dourson and Felter (1997), the proposed human equivalent oral doses of 0, 130, 940, or 2,700 mg/kg-day were used to estimate a BMDL for methyl t-butyl ether based on the incidence of Leydig cell tumors in male rats inhaling methyl t-butyl ether for two years (Appendix A Section 14.3). At human

equivalent oral doses of 0, 130, 940, and 2,700 mg/kg-day, the incidence of Leydig cell tumors was 32/50, 35/50, 41/50, and 47/50, respectively.

The Gamma, Multistage, Quantal Linear, and Weibull models provided the best fits, based on the same lowest AIC value, local Chi^2 values of less than the absolute value of two for all data points, and a good fit of both the BMD and BMDL dose-response plots (Appendix A). The calculated BMD (158.8 mg/kg-day) and BMDL (102.4 mg/kg-day) for all four models were identical and in close approximation of each other. As an alternate to a 10^{-5} cancer risk level for methyl t-butyl ether based on Leydig tumors in male rats or lymphatic tumors after gavage exposure, the BMDL of 102.4 mg/kg-day, based on Leydig tumors after inhalation exposure, can be used to calculate the 10^{-5} cancer risk level for methyl t-butyl ether.

$$10^{-5} \text{ risk level in rats} = \frac{\text{BMDL (0.00001)}}{0.1}$$

$$10^{-5} \text{ risk level in rats} = \frac{\text{102.4 mg/kg-day (0.00001)}}{0.1}$$

$$10^{-5} \text{ risk level in rats} = 0.01 \text{ mg/kg-day}$$

This 10^{-5} risk level dose of 0.01 mg/kg-day based on Leydig tumors in rats after inhalation exposure is three times higher than the 10^{-5} risk level dose of 0.003 mg/kg-day based on Leydig tumors in rats after gavage exposure.

Similarly, the proposed human equivalent oral doses of 0, 130, 940, or 2,700 mg/kg-day from Dourson and Felter (1997) were used to estimate a BMDL for methyl t-butyl ether based on the combined incidence of renal tubule adenoma and carcinomas in male rats observed after chronic inhalation. As suggested by OEHHA (1999), this approach considers that the renal tubule tumors are not associated with α-2μ-globulin nephropathy (Appendix A, Section 14.4). At human equivalent oral doses of 0, 130, 940, and 2,700 mg/kg-day, the combined incidence of renal tubule adenoma and carcinomas was 1/35, 0/32, 8/31, and 3/21, respectively.

Since none of the models provided a good fit, data for the highest dose level were omitted and the models were re-run (Appendix A, Section 14.5). The results of the BMDL modeling with the highest dose omitted provided a better fit than with all the dose levels. In general, however, the fits for all models using the data for renal tubule tumors in male rats after inhalation exposure were not as good as when using the data for the Leydig tumors in male rats after inhalation exposure.

When data for the highest dose level were omitted, the Multistage and Quantal Quadratic models provided the best fits, based on the same lowest AIC value, local Chi^2 values of less than the absolute value of two for all data points, and a good fit of both the BMD and BMDL dose-response plots (Appendix A). Since the calculated BMD (580 mg/kg-day) and BMDL (439 mg/kg-day) for the Quantal Quadratic model were in closer approximation of each other, the BMDL from the Quantal Quadratic model of 439 mg/kg-day was preferred over the BMDL of 303 mg/kg-day from the Multistage model. As an alternate to a 10^{-5} cancer risk level for methyl

t-butyl ether based on Leydig tumors in male rats after gavage exposure or Leydig tumors in male rats after inhalation exposure, the BMDL of 439 mg/kg-day, based on combined incidence of renal tubule adenoma and carcinomas in male rats after inhalation exposure, can be used to calculate the 10^{-5} cancer risk level for methyl t-butyl ether.

$$10^{-5} \text{ risk level in rats} = \frac{\text{BMDL } (0.00001)}{0.1}$$

$$10^{-5} \text{ risk level in rats} = \frac{439 \text{ mg/kg-day } (0.00001)}{0.1}$$

$$10^{-5} \text{ risk level in rats} = 0.04 \text{ mg/kg-day}$$

This 10^{-5} risk level dose of 0.04 mg/kg-day, based on renal tubule adenomas and carcinomas in rats after inhalation exposure, is more than one order of magnitude higher than the 10^{-5} risk level dose of 0.003 mg/kg-day, based on Leydig tumors in rats after gavage exposure, and four times higher than the 10^{-5} risk level dose of 0.01 mg/kg-day, based on Leydig tumors in rats after inhalation exposure. Overall, data for the Leydig tumors in male rats after inhalation exposure in the Chun et al. (1992) study provided the best fit for the BMDL models as compared to the data for Leydig tumors after gavage exposure from the Belpoggi et al. (1995; 1997; 1998) study or the renal tumors after inhalation exposure in the Chun et al. (1992) study.

However, recognizing the deficiencies in the Belpoggi et al. (1995) study, using a chronic oral study to estimate lifetime cancer risk from oral exposure to methyl t-butyl ether was considered more appropriate than using a chronic inhalation study and conducting an inhalation-route-to-oral-route extrapolation to estimate a lifetime cancer risk from oral exposure to methyl t-butyl ether. Although there are no chronic oral data in humans, there is *"suggestive evidence of carcinogenic potential"* after chronic gavage exposure to methyl t-butyl ether in rats. Further, the weight of genotoxicity evidence suggests that methyl t-butyl ether has some genotoxic potential and there were insufficient data to support a non-genotoxic mode of action. In the absence of mode of action information, the U.S. EPA (2003) generally takes a conservative, or public health-protective, default position, which assumes that the animal tumor findings are relevant to humans, and cancer risks are assumed to conform to low dose linearity. Based on this approach, the Leydig cell tumors in rats were assumed to be relevant to humans and the associated cancer risks were assumed to conform to low dose linearity. Thus, a 10^{-5} cancer risk level for methyl t-butyl ether was extrapolated from the chronic gavage $BMDL_{10}$ of 32 mg/kg-day for the Leydig cell tumors in male rats, which was essentially the same as the $BMDL_{10}$ of 36 mg/kg-day for leukemias/lymphomas (combined) in female rats. The Belpoggi et al. (1995) study was considered adequate for the purposes of risk assessment. The drinking water action levels developed in this risk assessment are protective of public health, since they were based on the tumor incidences observed in a chronic gavage study.

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Ward Jr., JB, D.H. Daiker, D.A. Hastings, M.M. Ammenheuser, and M.S. Legator. 1995. Assessment of the mutagenicity of methyl tertiary-butyl ether at the HPRT gene in CD-1 mice [abstract]. Toxicol. 15:79. As cited in OEHHA (1999) and ATSDR (1996).

Williams, T.M. and S.J. Borghoff. 2000. Induction of testosterone biotransformation enzymes following oral administration of methyl tert-butyl ether to male Sprague-Dawley rats. Toxicol. Sci. 57(1):147-55.

Williams, T.M., R.C. Cattley, and S.J. Borghoff. 2000. Alterations in endocrine responses in male Sprague-Dawley rats following oral administration of methyl tert-butyl ether. Toxicol. Sci. 54(1):168-76.

Yuan, Y., H.F. Wang, H.F. Sun, H.F. Du, L.H. Xu, Y.F. Liu, X.F. Ding, D.P. Fu, and K.X. Liu. 2007. Adduction of DNA with MTBE and TBA in mice studied by accelerator mass spectrometry. Environ Toxicol. 22(6):630-5.

Zhou,W. D. Yuan, G. Huang, H. Zhang, and S. Ye. 2000. Mutagenicity of Methyl Tertiary Butyl Ether. J. Environ. Pathol., Toxicol. Oncol. 19(1-2):35-39.

Zhou,W. and S. Ye. 1999. Subchronic oral methyl tertiary-butyl ether exposure (MTBE) in male Sprague-Dawley rats and effects on health of MTBE exposed workers. J. Occup. Health. 41:33-38.

13.0 APPENDIX A

13.1 Benchmark Dose for Leydig Cell Tumors after Gavage Exposure

```
================================================================================
        Gamma Model. (Version: 2.11;  Date: 10/31/2007)
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                  Thu Jan 24 13:29:50 2008
================================================================================
BMDS MODEL RUN
--------------------------------------------------------------------------------
  The form of the probability function is:

  P[response]= background+(1-background)*CumGamma[slope*dose,power],
  where CumGamma(.) is the cummulative Gamma distribution function

  Dependent variable = COLUMN2
  Independent variable = COLUMN1
  Power parameter is restricted as power >=1

  Total number of observations = 3
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

            Default Initial (and Specified) Parameter Values
              Background =     0.12963
                 Slope =   0.00333091
                 Power =          1.3

        Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s)  -Power have been estimated at a boundary point, or have been specified by
the user, and do not appear in the correlation matrix )

            Background      Slope
Background           1      -0.58

     Slope       -0.58          1

                    Parameter Estimates

                                                95.0% Wald Confidence Interval
    Variable      Estimate      Std. Err.   Lower Conf. Limit   Upper Conf. Limit
  Background      0.122284     0.0572279        0.0101192           0.234448
       Slope   0.00175848   0.000886228      2.15052e-005          0.00349546
       Power             1            NA
```

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-42.4001	3			
Fitted model	-42.4251	2	0.0499075	1	0.8232
Reduced model	-44.6509	1	4.50163	2	0.1053

AIC: 88.8501

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.1223	3.179	3	26	-0.107
42.5000	0.1855	4.637	5	25	0.187
170.0000	0.3491	11.171	11	32	-0.063

Chi^2 = 0.05 d.f. = 1 P-value = 0.8224

Benchmark Dose Computation

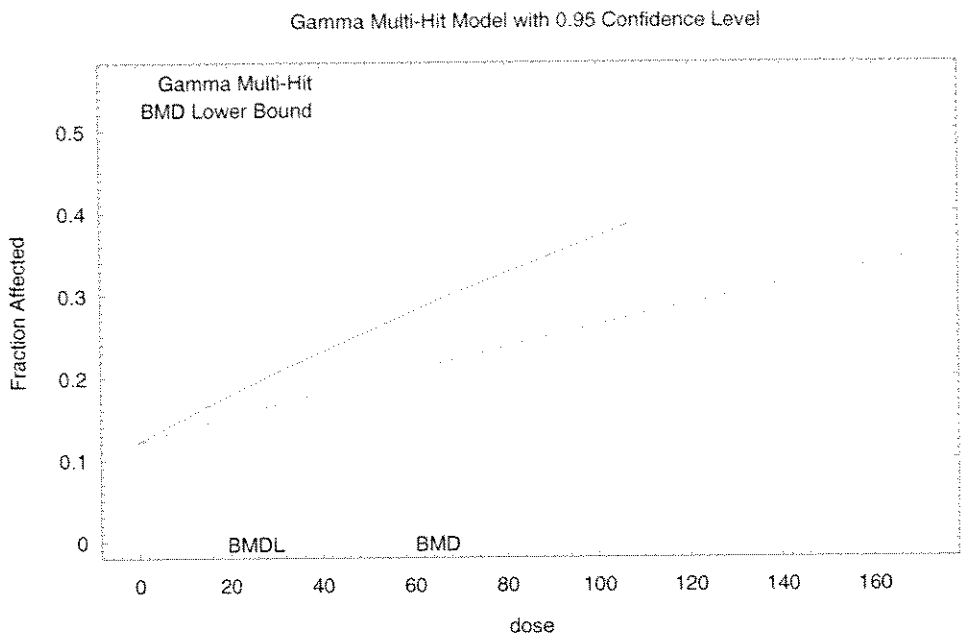
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 59.9156

BMDL = 31.5687



13.2 Benchmark Dose for Lymphomas/Leukemias after Gavage Exposure

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```
=============================================================================
        Gamma Model. (Version: 2.11;  Date: 10/31/2007)
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Jan 24 13:52:15 2008
=============================================================================
BMDS MODEL RUN
-----------------------------------------------------------------------------
  The form of the probability function is:

  P[response]= background+(1-background)*CumGamma[slope*dose,power],
  where CumGamma(.) is the cumulative Gamma distribution function

  Dependent variable = COLUMN2
  Independent variable = COLUMN1
  Power parameter is restricted as power >=1

  Total number of observations = 3
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
Background = 0.0423729
Slope = 0.00460149
Power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.49
Slope	-0.49	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.0419587	0.0264021	-0.00978844	0.0937059
Slope	0.00184323	0.00061981	0.000628423	0.00305803
Power	1	NA		

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.798	3			
Fitted model	-56.1041	2	0.612346	1	0.4339
Reduced model	-61.6305	1	11.665	2	0.002931

AIC: 116.208

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0420	2.434	2	58	-0.284
38.7000	0.1079	5.504	7	51	0.675
152.0000	0.2761	12.974	12	47	-0.318

Chi^2 = 0.64 d.f. = 1 P-value = 0.4246

Benchmark Dose Computation

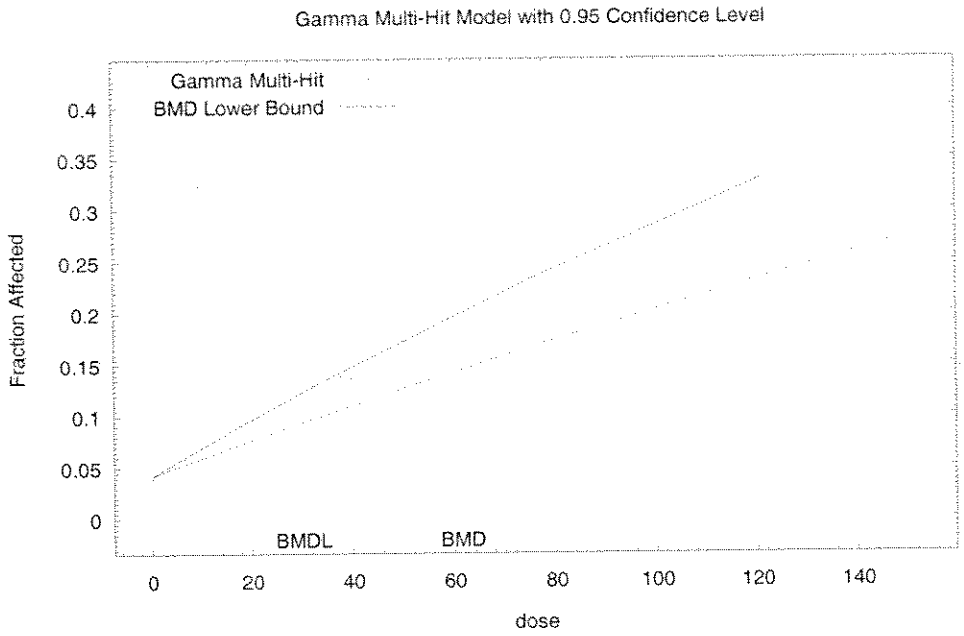
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 57.1609

BMDL = 35.7657



13.3 Benchmark Dose for Leydig Cell Tumors after Inhalation Exposure

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Results of benchmark dose modeling of Leydig cell tumors from male rats inhaling methyl t-butyl ether for two years

Model	Local Chi[2] scaled residuals <[2]?	p-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Gamma	Yes	0.9391	200.39	158.8	102.4
Logistic	Yes	0.6394	202.485	188.7	37.9
Multistage[c]	Yes	0.9391	200.39	158.8	102.4
Probit	Yes	0.8460	200.599	292.7	177.8
Quantal Linear	Yes	0.9391	200.39	158.8	102.4
Quantal Quadratic	Yes	0.3745	202.28	652.5	503.5
Weibull	Yes	0.9391	200.39	158.8	102.4

[a] p value should be greater than 0.1
[b] Graph was considered a good visual fit if the standard deviation for each dose fell within the estimated dose response curve
[c] Multistage degree of polynomial = 3
Note that Benchmark Dose Software Version 1.3.1 used

```
================================================================================
        $Revision: 2.2 $ $Date: 2001/03/14 01:17:00 $
        Input Data File: C:\BMDS\DATA\MTBE_CHUN_LEYDIG.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\MTBE_CHUN_LEYDIG.plt
                                                    Fri Nov 07 15:47:21 2003
================================================================================

BMDS MODEL RUN
--------------------------------------------------------------------------------

  The form of the probability function is:

  P[response]= background+(1-background)*CumGamma[slope*dose,power],
  where CumGamma(.) is the cummulative Gamma distribution function

  Dependent variable = COLUMN3
  Independent variable = COLUMN1
  Power parameter is restricted as power >=1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

            Default Initial (and Specified) Parameter Values
              Background =     0.637255
                 Slope =   0.00109377
                 Power =          1.3

       Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s)  -Power have been estimated at a boundary point, or have been specified by the
       user, and do not appear in the correlation matrix )

            Background      Slope
Background           1      -0.48
     Slope       -0.48          1

                  Parameter Estimates
      Variable        Estimate          Std. Err.
    Background        0.656225          0.0492578
       Slope       0.000663687       0.000200204
       Power             1              NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard
error.
                  Analysis of Deviance Table

      Model     Log(likelihood)  Deviance  Test DF    P-value
    Full model       -98.1322
   Fitted model      -98.195     0.125725     2        0.9391
  Reduced model      -106.633     17.0012     3      0.0007063

         AIC:         200.39
```

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.6562	32.811	32	50	-0.2415
130.0000	0.6846	34.232	35	50	0.2337
940.0000	0.8158	40.789	41	50	0.07694
2700.0000	0.9427	47.136	47	50	-0.08262

Chi-square = 0.13 DF = 2 P-value = 0.9391

Benchmark Dose Computation

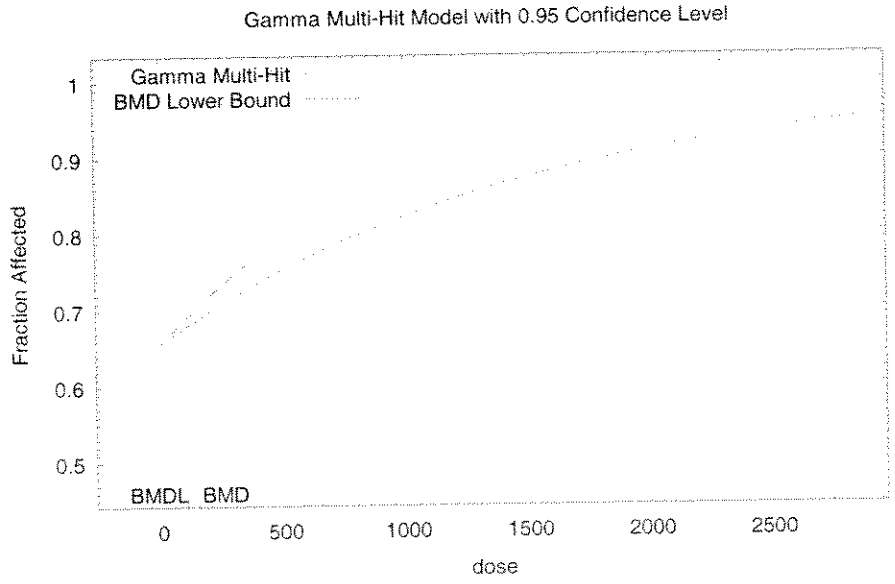
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 158.75

BMDL = 102.395



15:47 11/07 2003

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13.4 Benchmark Dose for Renal Tumors after Inhalation Exposure

Results of benchmark dose modeling of renal tubule adenomas and carcinomas (combined) from male rats inhaling methyl t-butyl ether for two years

Model	Local Chi² scaled residuals <[2]?	p-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Gamma	No	0.0136	74.7039	922	536
Logistic	No	0.0210	74.1405	816	444
Multistage[c]	Yes	0.0136	74.7039	922	538
Probit	No	0.0010	79.0339	1132	735
Quantal Linear	No	0.0136	74.7039	922	536
Quantal Quadratic	Yes	0.0005	79.8644	2108	1249
Weibull	No	0.0136	74.7039	922	536

[a] p value should be greater than 0.1
[b] Graph was considered a good visual fit if the standard deviation for each dose fell within the estimated dose response curve
[c] Multistage degree of polynomial = 3
Note that Benchmark Dose Software Version 1.3.1 used

Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
Input Data File: C:\BMDS\DATA\MTBE_CHUN_RENAL_OMIT_HIGH.(d)
Gnuplot Plotting File: C:\BMDS\DATA\MTBE_CHUN_RENAL_OMIT_HIGH.plt
Mon Nov 10 11:00:55 2003

BMDS MODEL RUN

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = COLUMN3
Independent variable = COLUMN1

Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0416667
Slope = 3.0124e-007
Power = 2 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.15

Slope -0.15 1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.015063	0.0149491
Slope	3.13042e-007	1.18417e-007

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-22.2426			
Fitted model	-23.0703	1.65528	1	0.1982
Reduced model	-30.0632	15.6411	2	0.0004014

AIC: 50.1405

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0151	0.527	1	35	0.6561
130.0000	0.0203	0.648	0	32	-0.8135
940.0000	0.2531	7.845	8	31	0.06399

Chi-square = 1.10 DF = 1 P-value = 0.2951

Benchmark Dose Computation

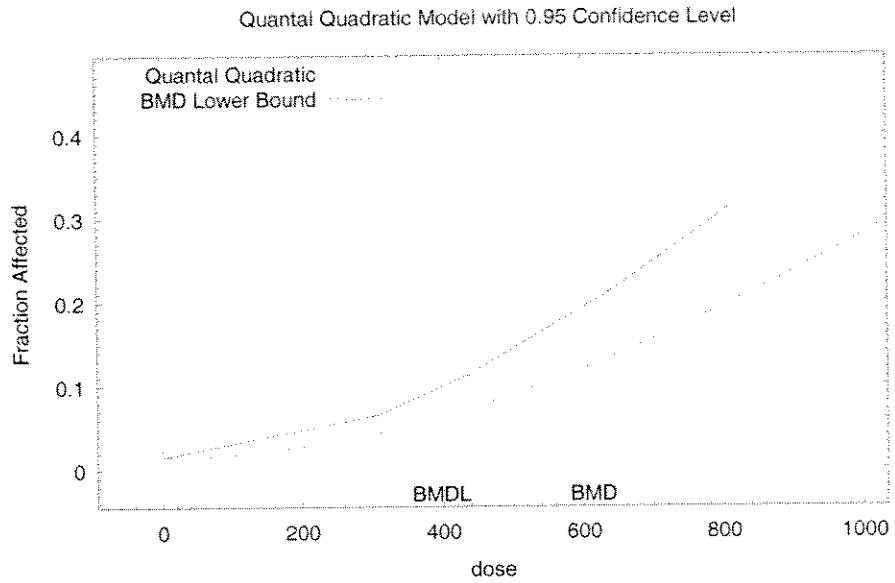
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 580.147

BMDL = 439.007



13.5 Benchmark Dose for Renal Tumors after Inhalation Exposure (omit highest dose)

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Results of benchmark dose modeling of renal tubule adenomas and carcinomas (combined) from male rats inhaling methyl t-butyl ether for two years (omit highest dose)

Model	Local Chi² scaled residuals <[2]?	p-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Gamma	Yes	NA	51.7978	773	323
Logistic	Yes	NA	51.7978	833	314
Multistage[c]	Yes	0.2951	50.1405	580	303
Probit	Yes	NA	51.7978	743	363
Quantal Linear	Yes	0.1422	52.1041	413	235
Quantal Quadratic	Yes	0.2951	50.1405	580	439
Weibull	Yes	NA	51.7978	839	324

[a] p value should be greater than 0.1
[b] Graph was considered a good visual fit if the standard deviation for each dose fell within the estimated dose response curve
[c] Multistage degree of polynomial = 3
Note that Benchmark Dose Software Version 1.3.1 used

```
=============================================================
        Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
        Input Data File: C:\BMDS\DATA\MTBE_CHUN_RENAL_OMIT_HIGH.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\MTBE_CHUN_RENAL_OMIT_HIGH.plt
                                        Mon Nov 10 11:00:55 2003
=============================================================

BMDS MODEL RUN
-------------------------------------------------------------
```

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = COLUMN3
Independent variable = COLUMN1

Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0416667
Slope = 3.0124e-007
Power = 2 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.15
Slope	-0.15	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.015063	0.0149491
Slope	3.13042e-007	1.18417e-007

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-22.2426			
Fitted model	-23.0703	1.65528	1	0.1982
Reduced model	-30.0632	15.6411	2	0.0004014

AIC: 50.1405

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0151	0.527	1	35	0.6561
130.0000	0.0203	0.648	0	32	-0.8135
940.0000	0.2531	7.845	8	31	0.06399

Chi-square = 1.10 DF = 1 P-value = 0.2951

Benchmark Dose Computation

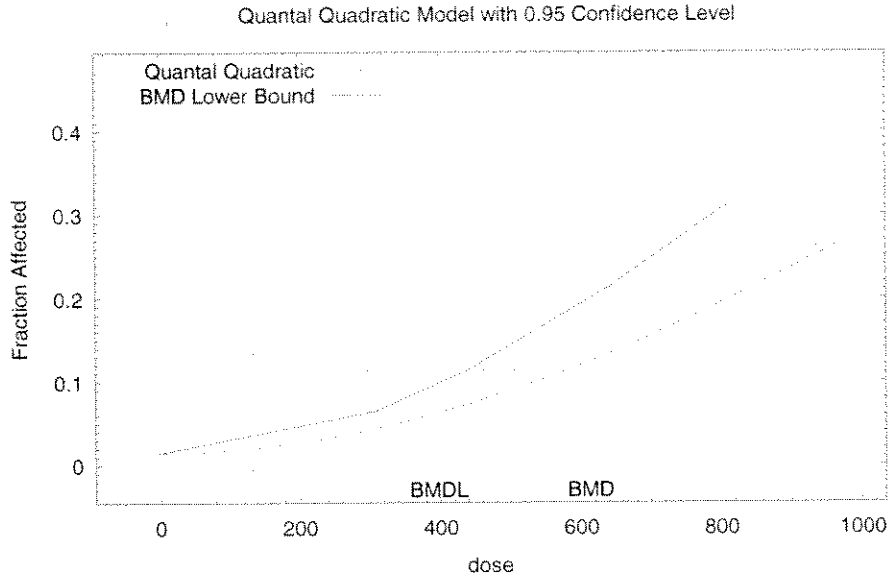
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 580.147

BMDL = 439.007



11:00 11/10 2003

13.0 PEER REVIEW HISTORY

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This document has not undergone external peer review.